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(54) Title: THERMOSTABLE CHIMERIC NUCLEIC ACID POLYMERASES AND USES THEREOF

(57) Abstract: Novel thermostable chimeric nucleic acid polymerases and methods for their generation and use are disclosed. It is shown that these chimeric nucleic acid polymerases, such as DNA polymerases, can be constructed using enzymatically active domains, isolated from different proteins or chemically synthesized. It is demonstrated that chimeric nucleic acid polymerases of the present invention possess the chemical and physical properties of their component domains (e.g., exonuclease activity, thermostability) and that the chimeric polymerases are thermostable.

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# THERMOSTABLE CHIMERIC NUCLEIC ACID POLYMERASES AND USES THEREOF

## Cross Reference to Related Applications

This application claims the benefit of U.S. Nonprovisional Application No. 09/506,153, filed 17 February 2000.

## Field of the Invention

The present invention is in the field of molecular biology. The present invention is directed to novel thermostable chimeric enzymes useful for the generation of nucleic acids, methods for making thermostable chimeric nucleic acid polymerases, and methods useful for polymerizing nucleic acids using a thermostable chimeric nucleic acid polymerase. Specifically, the invention is directed to chimeric thermostable DNA polymerases and their uses.

## Background of the Invention

Nucleic acid polymerases are an important class of compounds that enzymatically link (polymerize) nucleotides to form larger polynucleotide chains (e.g., DNA or RNA strands). Nucleic acid polymerases typically utilize a template polynucleotide (in either a single-strand or double-strand form) for nucleic acid synthesis, as in conventional nucleic acid replication, transcription, or reverse transcription. Other nucleic acid polymerases, e.g., terminal transferase (TdT), are capable of *de novo* polymerization, that is, template independent nucleic acid synthesis.

All known nucleic acid polymerases possess an enzymatic domain that catalyzes the formation of a phosphodiester bond between two nucleotides, utilizing the 5' carbon triphosphate of one nucleotide and the 3' carbon hydroxyl group of another nucleotide. Nucleic acid polymerases synthesize nascent polynucleotides by linking the 5' phosphate of one nucleotide to the 3' OH group of the growing polynucleotide strand. This process is known and commonly referred to by persons skilled in the art as 5'-3' polymerization.

In addition, nucleic acid polymerases possess a wide range of ancillary chemical properties useful for nucleic acid synthesis. These properties include, but are not limited to:

- product and/or template specificity (e.g., RNA or DNA);
- single-strand or double-strand template specificity;

- processivity - a measure of the ability of a nucleic acid polymerase to generate a nascent polynucleotide from a template polynucleotide without dissociating from the template;
- extension rate - a measure of the rate at which nucleotides are added to a growing polynucleotide strand;
- fidelity - a measure of the accuracy (or conversely the error rate) with which a nucleic acid polymerase synthesizes a polynucleotide complementary to a template polynucleotide;
- nick translation - the ability of a nucleic acid polymerase to degrade the preceding nucleotide strand of a double strand molecule simultaneous to polymerizing a nascent strand;
- proofreading - the ability of a nucleic acid polymerase to remove an incorrectly linked nucleotide from a polynucleotide before further polymerization occurs; and
- thermostability - the ability of a nucleic acid polymerase to retain activity after exposure to elevated temperatures.

Many of these properties are the result of one or more discrete functional domains within a polymerase holoenzyme. Three extensively studied enzymatically active domains of nucleic acid polymerase include: a 5'-3' polymerase domain, responsible for polynucleotide synthesis; a 5'-3' exonuclease domain, responsible for polynucleotide digestion of the 5' end of a polynucleotide, useful for nick translation; and a 3'-5' exonuclease domain, responsible for polynucleotide digestion of the 3' end of a polynucleotide, allowing for proofreading, and thus improving the fidelity of the polymerase. Some studies indicate that selection, incorporation, and extension of the correct nucleotide, versus an incorrect nucleotide, is a variable property of the 5'-3' polymerase domain, thus affecting polymerase fidelity in concert with proofreading activity (Mendelman et al., 1990; Petruska et al., 1988).

DNA polymerases can be categorized into six families based on amino acid homology. These families consist of; pol I, pol α,SONDZEICHEN pol β, SONDZEICHEN DNA-dependent RNA polymerase, reverse transcriptase, and RNA-dependent RNA polymerase (Joyce and Steitz, 1994). Table 1 summarizes the enzymatic features of a few representative DNA polymerases.

**Table 1.** DNA polymerase enzymatic activity

| DNA polymerase | (N terminus ---------- C terminus) 5'-3' exonuclease | 3'-5' exonuclease | 5'-3' polymerase | Thermo-stability | de novo polymerase |
|---|---|---|---|---|---|
| *E. coli* pol I | (+) | (+) | (+) | (-) | (-) |
| Klenow fragment | (-) | (+) | (+) | (-) | (-) |
| *E. coli* pol II | (-) | (+) | (+) | (-) | (-) |
| *E. coli* pol III | (+) | (+) | (+) | (-) | (-) |
| T4 pol | (-) | (+) | (+) | (-) | (-) |
| T7 pol | (-) | (+) | (+) | (-) | (-) |
| M-MuLV RT | (-) | (-) | (+) | (-) | (-) |
| TdT | (-) | (-) | (+) | (-) | (+) |
| *Taq* pol | (+) | (-) | (+) | (+) | (-) |
| Stoffel fragment | (-) | (-) | (+) | (+) | (-) |
| *Tbr* pol | (+) | (-) | (+) | (+) | (-) |
| *Tli* pol | (-) | (+) | (+) | (+) | (-) |
| *Tma* pol | (-) | (+) | (+) | (+) | (-) |
| *Tth* pol | (+) | (-) | (+) | (+) | (-) |
| *Pfu* pol | (-) | (+) | (+) | (+) | (-) |
| *Psp* pol | (-) | (+) | (+) | (+) | (-) |
| *Pwo* pol | (-) | (+) | (+) | (+) | (-) |

Because of the diversity of properties and characteristics potentially exhibited by nucleic acid polymerases generally, practitioners in the art have sought to modify, to alter, or to recombine various features of nucleic acid polymerases in an effort to develop new and useful variants of the enzyme. Initially, polymerase truncations and deletions were developed. The Klenow fragment, for example, was the first nucleic acid polymerase variant developed. Klenow fragments exist as a large C-terminal truncation of DNA polymerase I (pol I), possessing an enzymatically active 3'-5' exonuclease and 5'-3' polymerase domains, but lacking altogether the 5'-3' exonuclease domain of native pol I (Klenow and Henningsen, 1970; Jacobson et al., 1974; and Joyce and Grindley, 1983).

Since the advent of the polymerase chain reaction (PCR) methodology (including derivative methodologies such as reverse transcription PCR, or RT-PCR), resilient nucleic acid polymerases, capable of withstanding temperature spikes as high as 95°C without a subsequent significant loss in enzymatic activity (i.e., thermostable) have become vital tools in modern molecular biology. The use of thermostable enzymes to amplify nucleic acid sequences is described in U.S. Pat. Nos. 4,683,195 and 4,683,202. A thermostable DNA polymerase from *Thermus aquaticus* (*Taq*) has been cloned, expressed and purified from recombinant cells (Lawyer et al., 1989; U.S. Pat. Nos. 4,889,818 and 5,079,352. PCR is also described in many U.S. patents, including U.S. Pat. Nos. 4,965,188, 4,683,195, 4,683,202, 4,800,159, 4,965,188, 4,889,818, 5,075,216, 5,079,352, 5,104,792, 5,023,171, 5,091,310, and 5,066,584.).

As depicted in Table I, *Taq* DNA polymerase possesses enzymatically active 5'-3' polymerase and 5'-3' exonuclease domains, but it exhibits only background levels of 3'-5' exonuclease activity (Lawyer et al., 1989; Bernard et al., 1989; Longley et al., 1990). Crystallographic data revealed that *Taq* polymerase contains a 3'-5' exonuclease domain (Eom et al., 1996); comparisons of the crystal structure of the Klenow fragment from *Bacillus* DNA polymerase I, *Taq* DNA polymerase, and *E. coli* DNA polymerase I have shown, however, that critical residues required to carry out a 3'-5' exonuclease activity are missing in the 3'-5' exonuclease domain of *Taq* DNA polymerase (Kiefer et al., 1997). Park et al. (1997), have determined that *Taq* DNA polymerase possesses none of three sequence motifs (*Exo* I, II, and III) within the 3'-5' exonuclease domain and necessary for 3'-5' exonuclease activity. Because *Taq* polymerase exhibits essentially no 3'-5' exonuclease activity (i.e., proofreading capability), the error rate of *Taq* DNA polymerase is high compared to other DNA polymerases that possess an enzymatically active 3'-5' exonuclease domain (Flaman et al., 1994). The *Taq* DNA polymerase structure thus comprises a 5'-3' exonuclease domain occurring at the N-terminal region of the polypeptide (residues 1-291), followed by an enzymatically inactive 3'-5' exonuclease domain (residues 292-423), and a C-terminal 5'-3' polymerase domain (Park et al., 1997).

Since *Taq* DNA polymerase does not possess an enzymatically active 3'-5' exonuclease domain, providing a proofreading feature to the polymerase, the use of *Taq* DNA polymerase becomes less desirable for most nucleic acid amplification applications, e.g., for PCR sequencing protocols or amplification for protein expression, which require complete identity of replication products to the template nucleic acid. Depending on the phase of PCR during which an error becomes incorporated into the PCR product (e.g., in an early replication cycle), the entire population of amplified DNA could contain one or more sequence errors, giving rise to a nonfunctional and/or mutant gene product. Nucleic acid polymerases that possess an enzymatically active 3'-5' exonuclease domain (i.e., proofreading activity), therefore, are especially preferred for replication procedures requiring high fidelity.

Due to the scientific and commercial importance of PCR in modern molecular biology, the reliance of PCR protocols on nucleic acid polymerases of particular characteristics, and in view of the enzymatic deficiencies of *Taq* polymerase, an enormous amount of research and development has focussed on developing new and useful thermostable DNA polymerase variants and/or assemblages.

One approach has been directed to the discovery and isolation of new thermophilic nucleic acid polymerases, which may possess a unique and/or improved collection of catalytic properties. As a result, thermostable nucleic acid polymerases have been isolated from a variety of biological sources, including, but not limited to, species of the

taxonomic genera, Thermus, Thermococcus, Thermotoga, Pyrococcus, and Sulfolobus. These polymerases possess a variety of chemical characteristics, as illustrated in Table 1. Some of these naturally occurring thermostable DNA polymerases possess enzymatically active 3'-5' exonuclease domains, providing a natural proofreading capability and, thus, exhibiting higher fidelity than *Taq* DNA polymerase. Naturally occurring proofreading thermostable polymerases include: *Pfu* polymerase (isolated from *Pyrococcus furiosus*), *Pwo* polymerase (isolated from *Pyrococcus woesei*), *Tli* polymerase (isolated from *Thermococcus litoralis*), and *Psp* polymerase (isolated from *Pyrococcus* sp. GB-D). All of these naturally occurring thermostable polymerases are commercially available (*Tli* polymerase and *Psp* polymerase are marketed as Vent® and Deep Vent$^{SONDZEICHEN®}$ DNA polymerase, respectively, by New England Biolabs, Beverly, MA). These DNA polymerases show slower DNA extension rates and an overall lower processivity when compared to *Taq* DNA polymerase, however, thus rendering these naturally occurring thermostable DNA polymerases less desirable for PCR, despite their higher fidelity.

In an effort to compensate for the deficiencies of individual thermostable polymerases, a second approach has been to develop multiple enzyme assemblages, combining, for example, *Taq* polymerase and a proofreading enzyme, such as *Pfu* polymerase or Vent$^{SONDZEICHEN®}$ polymerase. These multiple-enzyme mixtures exhibit higher PCR efficiency and reduced error rates when compared to *Taq* polymerase alone (Barnes, 1994). Mixtures of multiple thermostable enzymes are commercially available (e.g., the Failsafe™ PCR system from Epicentre, Madison, WI). PCR protocols utilizing multiple polymerase mixtures are still prone to error, however, and require the practitioner to perform preliminary experimental trials, to determine special optimized solution conditions necessary for multiple-enzyme reaction mixtures.

A third approach has been to develop new and useful variants of *Taq* polymerase through deletion/truncation techniques. The Stoffel fragment, for example, is a 544 amino acid C-terminal truncation of *Taq* DNA polymerase, possessing an enzymatically active 5'-3' polymerase domain but lacking 3'-5' exonuclease and 5'-3' exonuclease activity. Other commercially available thermostable polymerase deletions include Vent$^{SONDZEICHEN®}$ (exo$^-$) and Deep Vent$^{SONDZEICHEN®}$ (exo$^-$) (New England Biolabs, Beverly , MA). Deletion mutations serve only to remove functional domains of a nucleic acid polymerase, however, and do not add any novel features or enzymatic properties.

Polymerase mutagenesis is yet another approach that has been attempted to develop new and useful nucleic acid polymerase variants. Park et al. (1997) performed site-directed mutagenesis of 4 amino acids in the enzymatically inactive 3'-5' exonuclease domain of *Taq* polymerase in an effort to activate the proofreading ability of this domain.

The resultant mutant exhibited an increase of exonuclease activity over that of naturally occurring *Taq* polymerase. The reported increase was a mere two-fold increase above background exonuclease activity, however; an insignificant rise in exonuclease activity that is unlikely to increase PCR fidelity.

Bedford et al. (1997) developed a recombinant mesophilic DNA pol I from *E. coli*. They succeeded to insert a thioredoxin binding domain from T7 DNA polymerase into *E. coli* pol I. The inserted 76 amino acid binding domain improved polymerase binding to a template polynucleotide, thus increasing the processivity of the recombinant *E. coli* pol I but did not improve or provide any novel enzymatic activity to the polymerase.

Recently Gelfand et al. (1999) combined fusion protein technology with mutagenesis to eliminate or substantially reduce 5'-3' exonuclease activity and 3'-5' exonuclease activity in recombinant polymerases. Once again, no improved or additional enzymatic activity was provided by the fusion polymerase.

Frey et al. (1999) attempted to engineer chimeric polymerases utilizing enzymatically active domains from *Taq*, *Tne*, and *E. coli* DNA polymerases. Although they successfully substituted the non-functional 3'-5' exonuclease domain of *Taq* DNA polymerase with a functional 3'-5' exonuclease domain from another DNA polymerase, their resultant chimeric polymerase lost significant, if not all, enzymatic activity after only one minute at 80°C or 95°C (i.e., they are not thermostable), and thus are not useful for performing PCR protocols without the successive addition of fresh polymerase for each cycle.

Despite these intense research efforts, there remains a need in the art for thermostable nucleic acid polymerases that possess improved or novel assemblages of enzymatically active domains. Despite its enzymatic deficiencies, *Taq* DNA polymerase remains the most widely used enzyme for processing *in vitro* amplification of nucleic acids. In particular, there has been long felt need for a nucleic acid polymerase possessing the 5'-3' polymerization qualities of *Taq* polymerase, but which also possesses 3'-5' exonuclease (proofreading) activity.

## Summary of the Invention

In response to the long felt need for new and useful nucleic acid polymerases, a novel approach for producing thermostable nucleic acid polymerases was invented. The present invention represents the first thermostable chimeric nucleic acid polymerase, useful for continuous PCR protocols, obtained by combining at least two enzymatically active domains from different proteins by means of recombinant DNA techniques.

The present invention is directed to novel thermostable chimeric enzymes useful for the generation of nucleic acids, methods for making thermostable chimeric nucleic acid

polymerases, and methods useful for polymerizing nucleic acids using a thermostable chimeric nucleic acid polymerase. The thermostable chimeric nucleic acid polymerase of the present invention comprises at least two enzymatically active domains, which are non-naturally associated. The recombinant association of the enzymatically active domains results in a composite enzyme not found in nature. The thermostable chimeric nucleic acid polymerase of the present invention possesses new or improved catalytic properties compared to nucleic acid polymerases known in the art.

The thermostable chimeric nucleic acid polymerase of the present invention offers several advantages over previous approaches to develop novel nucleic acid polymerases. The present invention provides a single enzyme that possesses a suite of chemical properties, the combination of which may not necessarily exist in nature, but nonetheless is useful in molecular biology. The chimeric nucleic acid polymerase of the present invention eliminates the need to specifically develop multiple-enzyme reaction mixtures, which are often difficult to optimize and expensive to use, and the necessity to add successive amounts of fresh enzyme during each cycle of a PCR program. The invention thus facilitates the rapid, efficient, and accurate generation of nucleic acid molecules, particularly in regard to PCR protocols.

Definitions

As used herein, an "enzymatically active domain" refers to any polypeptide, naturally occurring or synthetically produced, capable of mediating, facilitating, or otherwise regulating a chemical reaction, without, itself, being permanently modified, altered, or destroyed. Binding sites (or domains), in which a polypeptide does not catalyze a chemical reaction, but merely forms noncovalent bonds with another molecule, are not enzymatically active domains as defined herein. In addition, catalytically active domains, in which the protein possessing the catalytic domain is modified, altered, or destroyed, are not enzymatically active domains as defined herein. Enzymatically active domains, therefore, are distinguishable from other (nonenzymatic) catalytic domains known in the art (e.g., detectable tags, signal peptides, alosteric domains, etc.).

As defined herein, a 3'-5' exonuclease domain refers to any polypeptide capable of enzymatically cleaving a nucleotide from the 3' end of a di- or polynucleotide, a 5'-3' exonuclease domain refers to any polypeptide capable of enzymatically cleaving a nucleotide from the 5' end of a di- or polynucleotide, and a 5'-3' polymerase domain refers to any polypeptide capable of enzymatically linking the 5' phosphate of one nucleotide to the 3' OH group of another nucleotide.

Polypeptide domains that are "non-naturally associated", refer to specific polypeptides that are not naturally produced within a single polypeptide; that is, the

polypeptide domains are not naturally translated from a common nucleic acid transcript in a naturally occurring organism. Non-naturally associated polypeptide domains include domains isolated from functionally distinct proteins, separately produced by an organism of one or more species, or synthetically generated, as well as polypeptide domains isolated from functionally similar proteins, but naturally produced by organisms of different species, or synthetically generated. The term "non-naturally associated polypeptide domains" refers to domains that are associated or fused only through human intervention; the term expressly excludes naturally occurring enzymes or fragments thereof.

As used herein, the term "chimeric protein" encompasses all proteins that contain two or more polypeptide domains that are non-naturally associated (regardless of whether the domains are naturally produced by organisms of the same species, different species, or synthetically generated). A chimeric nucleic acid polymerase of the present invention must necessarily possess two or more non-naturally associated domains, as defined herein.

The term "thermostable" generally refers to the resilience of a substance to relatively high temperature treatment. A thermostable enzyme is an enzyme that retains its definitive enzymatic activity despite exposure to relatively high temperature. A thermostable nucleic acid polymerase, as generally understood by practitioners in the art and as defined herein, refers to a polymerase that is useful for PCR protocols; i.e., not requiring successive or supplemental addition of enzyme after each high temperature step of the PCR program cycle. The chimeric nucleic acid polymerase of the present invention is thermostable, in that it is useful for PCR protocols, because it does not require successive or supplemental addition of polymerase after each high temperature step of the PCR program cycle.

A preferred thermostable chimeric polymerase of the present invention is one that allows a thermal polymerase chain reaction to proceed with only an initial supply of polymerase at the start of the PCR program. Preferably, a thermostable chimeric nucleic acid polymerase retains some measurable enzymatic activity at its normal operating temperature (typically about 72°C) after exposure to 95°C for three minutes. More preferably, a thermostable chimeric nucleic acid polymerase is able to withstand one minute at 95°C without significant loss (> 5% loss) in enzymatic activity. In other words, a preferred thermostable chimeric nucleic acid polymerase retains at least about 95% of its polymerase activity at its normal operating temperature (typically about 72°C) after one minute at 95°C. Even more preferably, a thermostable chimeric nucleic acid polymerase is able to withstand three minutes at 95°C without significant loss in enzymatic activity. A most preferred thermostable chimeric nucleic acid polymerase is able to withstand ten minutes at 90°C and still retain at least about 50% of its enzymatic activity at its normal

operating temperature. In other words, the polymerase displays a "half life" (the length of time it takes for a substance to lose one half of its initial activity) of ten minutes at 90°C. Ideally, a thermostable chimeric nucleic acid polymerase displays a half-life comparable to the half-life measurement of naturally occurring thermostable nucleic acid polymerases. For example a most desirable thermostable chimeric nucleic acid polymerase displays a half-life at 90°C comparable to that of Taq polymerase, approximately 90 minutes.

The present invention is directed generally to all thermostable chimeric nucleic acid polymerases comprising at least two non-naturally associated enzymatically active domains. As defined herein, a nucleic acid polymerase is any enzyme that catalyzes the formation of chemical bonds between (chemically bonds) nucleotides to form polynucleotide chains, that is, any enzyme that promotes nucleic acid polymerization. The thermostable chimeric nucleic acid polymerases of the present invention include all types of nucleic acid polymerases, without limitation to product or template specificity, molecular requirements, or chemical properties (e.g., RNA vs. DNA, single strand vs. double strand, high fidelity, etc.).

One embodiment of the present invention is directed to a thermostable chimeric DNA polymerase, preferably a chimeric DNA polymerase wherein the enzymatically active domains are isolated from naturally occurring proteins from two or more species, or any mutants, variants, or derivatives thereof.

As used herein, mutant, variant, and derivative polypeptides refer to all chemical permutations of a given polypeptide, which may exist or be produced, that still retain the characteristic molecular activity that is definitive of that polypeptide.

The thermostable chimeric nucleic acid polymerase of the present invention is unexpected in view of the fact that enzymatically active domains may be isolated from a wide variety of sources, yet still retain their enzymatic activities (e.g., polymerase, exonuclease) and chemical properties (e.g., thermostability, processivity). Enzymatically active domains isolated from organisms of different taxonomic kingdoms and from completely different families of proteins may be fused to produce an entirely novel, yet functional, nucleic acid polymerase. For example, enzymatically active domains from a eubacterium polymerase of e.g., *Taq* polymerase may be chimerically joined with enzymatically active domains from an archaeon polymerase (e.g., *Pwo*, *Sso*, and *Pho* polymerases).

Retention of thermal stability in a fusion protein engineered from different thermophilic proteins is highly unexpected. Attempts to construct chimeric polymerases have failed to produce thermostable chimeric polymerases (see Frey et al., 1999). The underlying principles of thermal stability of proteins derived from thermophilic organisms are not known. Even small changes in the amino acid sequence of thermoresistant

proteins result in a significant decrease in thermal stability and an associated reduction in enzymatic activity of the protein. Maintenance of, or an increase in, thermal stability of thermostable DNA polymerase has only been accomplished by truncation of a DNA polymerase (e.g., Barnes, 1995). The present invention represents the first chimeric nucleic acid polymerase, containing enzymatically active domains from different thermostable proteins, that possess thermostable properties.

In a preferred embodiment, at least one of the enzymatically active domains of the chimeric nucleic acid polymerase is isolated from a DNA polymerase produced by a thermophilic organism, preferably an organism of a genus selected from the group of genera consisting of: Thermus, Thermococcus, Thermotoga, Pyrococcus, Pyrodictium, Bacillus, Sulfolobus, and Methanobacterium. Most preferably, at least one of the enzymatically active domains of the chimeric nucleic acid polymerase is isolated from a DNA polymerase selected from the group consisting of: *Thermoplasma acidophilum (Tac)* polymerase; *Thermus aquaticus (Taq)* polymerase; *Thermococcus barossii (Tba)* polymerase; *Thermus brockianus (Tbr)* polymerase; *Tfi* polymerase; *Thermus flavus (Tfl)* polymerase; *Thermococcus litoralis (Tli)* polymerase; *Thermococcus pacificus (Tpac)* polymerase; *Thermus ruber (Tru)* polymerase; *Thermus thermophilus (Tth)* polymerase; *Pyrodictium abyssi (Pab)* polymerase; *Pyrococcus furiosus (Pfu)* polymerase; *Pyrococcus hellenicus (Phe)* polymerase; *Pyrococcus horikoshii (Pho)* polymerase; *Pyrococcus kodakarensis (Pko)* polymerase; Pyrococcus sp. strain KOD1 (KOD) polymerase; Pyrococcus sp. strain ES4 (ES4) polymerase; *Pyrodictium occultum (Poc)* polymerase; *Pyrococcus* sp. GB-D (*Psp)* polymerase; *Pyrococcus woesei (Pwo)* polymerase; *Thermotoga maritima (Tma)* polymerase; *Thermotoga neapolitana (Tne)* polymerase; *Bacillus sterothermophilus (Bst)* polymerase; *Sulfolobus acidocaldarius (Sac)* polymerase; *Sulfolobus solfataricus (Sso)* polymerase; *Methanobacterium thermoautotrophicum (Mth)* polymerase; and mutants, variants, and derivatives thereof.

In another embodiment of the invention, the enzymatically active domains are selected from the group consisting of: 5'-3' exonuclease domain, 3'-5' exonuclease domain, and 5'-3' polymerase domain. Preferably the enzymatically active domains are naturally occurring domains, isolated from two or more species, most preferably the enzymatically active domains are isolated from naturally occurring thermostable proteins, mutants, variants, or derivatives thereof.

Another aspect of the present invention relates to an isolated polynucleotide encoding a thermostable chimeric nucleic acid polymerase comprising at least two non-naturally associated enzymatically active domains. Preferably the enzymatically active domains are isolated from different species.

A related aspect of the invention is directed to a method for synthesizing a recombinant nucleic acid that encodes a thermostable chimeric nucleic acid polymerase comprising at least two non-naturally associated enzymatically active domains.

A further aspect of the invention relates to a vector comprising a polynucleotide that encodes a thermostable chimeric nucleic acid polymerase having at least two non-naturally associated enzymatically active domains. Preferred vectors are expression vectors, which will be suitable for production of the encoded chimeric nucleic acid polymerase in transformed host cells.

Another aspect of the invention includes a recombinant host cell transformed with a vector comprising a polynucleotide that encodes a thermostable chimeric nucleic acid polymerase possessing at least two non-naturally associated enzymatically active domains.

A related aspect of the invention is directed to a method for producing a thermostable chimeric nucleic acid polymerase comprising at least two non-naturally associated enzymatically active domains.

Another aspect of the invention is directed to a process of nucleic acid polymerization, which necessarily utilizes a thermostable chimeric nucleic acid polymerase having at least two non-naturally associated enzymatically active domains.

A related aspect of the invention is directed to a kit useful for polymerization of nucleic acid, comprising a thermostable chimeric nucleic acid polymerase having at least two non-naturally associated enzymatically active domains. Preferably, the kit further comprises at least one reagent suitable for nucleic acid polymerization. Most preferably, the kit further comprises at least one reagent selected from the group consisting of one or more additional enzymes, one or more oligonucleotide primers, a nucleic acid template, any one or more nucleotide bases, an appropriate buffering agent, a salt, or other additives useful in nucleic acid polymerization.

Brief Description of the Drawings

**Fig. 1** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which depicts the polymerase activity of thermostable chimeric DNA polymerases using a primer extension reaction. Lane 1 shows a nucleic acid ladder, used as a gel reference marker. Lanes 2, 6 and 10 show negative controls (without addition of polymerase). Lane 3, 4, 5 show the activity of 0.05, 0.03 and 0.01 units *Taq* DNA polymerase, respectively. Lanes 7-9 illustrate polymerase activity of undiluted cleared lysate, a 1:1, and 1:5 diluted cleared lysate, of a *Pho/Taq* chimeric polymerase, respectively.

**Fig. 2** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which depicts the thermostability of a thermostable chimeric DNA polymerase compared to *Taq*

DNA polymerase, using a primer extension reaction. DNA polymerases were incubated for various time spans at 90ºC and assayed for remaining polymerase activity. Lanes 1 and 11 show a nucleic acid ladder, used as a gel reference marker. Lanes 2, 10, 12, and 20 represent negative control reactions (without addition of polymerase). Lanes 3-9 and lanes 13-19 illustrate DNA polymerase activity after incubation of *Taq* DNA polymerase and a *Pho/Taq* chimeric DNA polymerase at 90ºC for 0, 10, 15, 30, 60, 90, and 120 min, respectively.

**Fig. 3** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which depicts 3'-5' exonuclease activity of three different thermostable DNA polymerases. **(A)** illustrates PCR product using a wild type primer combination. **(B)** illustrates PCR product using a mutant primer pair. Lane 1 is a nucleic acid ladder, used as a gel reference marker. The PCR amplification product of *Taq* DNA polymerase is shown in lanes 2-5; *Pfu* DNA polymerase I PCR product is shown in lanes 6-9; and a *Pho/Taq* thermostable chimeric DNA polymerase PCR product is shown in lanes 10-13. Duplicate side-by-side reactions are shown representing undigested (the first and third lane for each enzyme used), and digested (the second and fourth lane for each enzyme used) PCR product.

**Fig. 4** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which illustrates the combined effect of primer extension efficiency and polymerase processivity on PCR efficiency of three different thermostable DNA polymerases. The photograph illustrates PCR products obtained in duplicate reactions using different primer extension times. **(A)** indicates PCR products obtained with *Taq* DNA polymerase. **(B)** illustrates PCR products obtained with a *Pho/Taq* thermostable chimeric DNA polymerase. **(C)** shows PCR products generated with Pfu DNA polymerase I. Lane 1 is a nucleic acid ladder, used as a gel reference marker. Lanes 2-3 show PCR products amplified after primer extension for 1 min. Lanes 4-5 show PCR products amplified after primer extension for 30 sec. Lanes 6-7 show PCR products amplified after primer extension for 10 sec. Lanes 8-9 show PCR products amplified after primer extension for 5 sec.

Detailed Description of the Invention

Genetic engineering techniques were successfully employed to generate the first thermostable chimeric nucleic acid polymerase, containing enzymatically active domains, not naturally found within a single protein. The chimeric nucleic acid polymerase and methods described herein encompass all thermostable nucleic acid polymerases, without limitation to product or template specificity, molecular requirements, or chemical properties. For example, chimeric nucleic acid polymerases of the present invention include single or double strand DNA polymerases, RNA polymerases, and reverse transcriptases. Thermostable chimeric nucleic acid polymerases of the present invention

may possess any number and/or combination of properties and features including, but not limited to, template dependence or independence, high processivity, high fidelity, proofreading, nick translation, and high extension rates. Persons skilled in the art will understand and appreciate that these features are due, in large part, to the presence and characteristics of discrete polypeptide domains within the holoenzyme. Essential to the chimeric nucleic acid polymerase of the present invention is that it possess at least two enzymatically active domains that are not naturally associated, and the chimeric nucleic acid is thermostable.

Enzymatically active domains may be isolated from any natural polypeptide, or may be synthetically produced. Natural polypeptides include any polypeptide found in nature, and from any organism of any taxonomic group. Enzymatically active domains useful in the present invention also include variant, mutant, or derivative forms of domains found in nature. Enzymatically active domains further include domains that may not be found in nature, e.g., polypeptides randomly generated or engineered in the laboratory or selected from a non-naturally generated library of polypeptides. For the purposes of this invention, enzymatically active domains need only necessarily possess an enzymatic activity that is functional within the chimeric nucleic acid polymerase of the invention. The thermostable chimeric nucleic acid polymerases of the present invention specifically contemplates incorporation into a nucleic acid polymerase, enzymatically active domains that are absent, inactive, or weakly active in the naturally occurring protein.

Persons skilled in the art will know and appreciate that a wide variety of enzymatic domains exist that perform the same or similar enzymatic functions. For example, DNA polymerases possess 3'-5' exonuclease domains of a wide range of enzymatic functionality; from little or no 3'-5' exonuclease activity (as seen in *Taq* polymerase), to fully functional 3'-5' exonuclease activity (as seen in *E. coli* pol I), to thermostable 3'-5' exonuclease activity (as seen in *Pwo* polymerase). It is understood by practitioners in the art that enzymatically active domains of individual polymerases are considered separate and distinct enzymatically active domains, as defined herein. Thus, the incorporation of an enzymatically active domain from one polymerase into a second polymerase produces, by definition, a chimeric polymerase, regardless of whether the second polymerase naturally possesses its own enzymatically active domain of similar functionality.

Preferably, genetic engineering techniques may be used to generate novel thermostable DNA polymerases possessing either 5'-3' polymerase activity and 3'-5' exonuclease activity; or 5'-3' polymerase activity, 3'-5' exonuclease activity and 5'-3' exonuclease activity derived from different thermostable DNA polymerases, e.g. *Taq* polymerase, *Pho* polymerase, *Pwo* polymerase, *Sso* polymerase, and *Tpac* polymerase.

Preferred thermostable chimeric nucleic acid polymerases of the present invention include a 5'-3' polymerase domain of *Taq* polymerase. For example, the Stoffel fragment is a 544 residue N-terminal deletion of *Taq* polymerase possessing an enzymatically active 5'-3' polymerase domain and an enzymatically inactive 3'-5' exonuclease domain. Generally, a *Taq* 5'-3' polymerase domain is at least about 544 residues in length, and includes any mutant, variant, or derivative of the Stoffel fragment of *Taq* polymerase, as defined herein. A 552 amino acid polypeptide, residue numbers 281-832 of *Taq* polymerase (SEQ ID NO:1), is an especially preferred enzymatically active *Taq* 5'-3' polymerase domain useful in the present invention.

Alternatively, the thermostable chimeric nucleic acid polymerases of the present invention may include a 5'-3' polymerase domain of *Tth* polymerase. *Tth* polymerase is capable of reverse transcription. Thermostable chimeric nucleic acid polymerases, which include the *Tth* 5'-3' polymerase domain, therefore, may be used for reverse transcription reactions (e.g., RT-PCR). Preferably, the 5'-3' polymerase domain of *Tth* polymerase is about 562 residues in length, including residue numbers 273-834 of *Tth* polymerase (SEQ ID NO:2), and includes any mutant, variant, or derivative thereof.

Preferred thermostable chimeric nucleic acid polymerases of the present invention also include an enzymatically active 3'-5' exonuclease domain of a thermostable polymerase. Preferred 3'-5' exonuclease domains include the enzymatically active 3'-5' exonuclease domains of *Pho* polymerase, *Pwo* polymerase, *Sso* polymerase, and *Tpac* polymerase. Most preferred are residues 1-396 of *Pho* polymerase (SEQ ID NO:3), residues 1-396 of *Pwo* polymerase (SEQ ID NO:4), residues 1-421 of *Pwo* polymerase (SEQ ID NO:5), residues 1-508 of *Sso* polymerase (SEQ ID NO:6), residues 1-395 of *Tpac* polymerase (SEQ ID NO:16), and any mutants, variants, or derivatives of any one of these 3'-5' exonuclease domains, as defined herein.

A process for synthesizing a recombinant nucleic acid encoding a thermostable chimeric nucleic acid polymerase of the invention necessarily comprises isolating at least two nucleic acid fragments each encoding at least one enzymatically active domain, which is not naturally associated with the other enzymatically active domain (i.e., derived from separate polypeptides), and genetically combining the nucleic acids of the enzymatically active domains to form a chimeric nucleic acid.

For production of thermostable chimeric nucleic acid polymerases according to the invention, the nucleic acid encoding a chimeric nucleic acid polymerase may be stably inserted into a genetic vector, preferably the nucleic acid is operably inserted into an expression vector, and most preferably the vector construct is capable of replication within a host organism, such that the nucleic acid encoding a thermostable chimeric nucleic acid polymerase is capable of being transcribed and translated into a polypeptide. A preferred

mode of making the chimeric nucleic acid polymerase of the present invention includes culturing a host cell containing a nucleic acid encoding a thermostable chimeric nucleic acid polymerase under conditions suitable for expression of the chimeric nucleic acid polymerase by the host cell, and isolating the chimeric nucleic acid polymerase expressed from said cell culture.

Methods for generating recombinant nucleic acids, vector construction, host cell transformation, and polypeptide expression systems useful in the practice of this invention can involve a wide variety of modern genetic engineering techniques, tools, and biological sources that are well known in the art and routinely practiced by those skilled in the art. Exemplary techniques and methods are described in detail herein by way of preferred example, but are not limiting to the practice of the invention. The present invention incorporates by reference in their entirety techniques and supplies well known in the field of molecular biology, including, but not limited to, techniques and supplies described in the following publications:

Ausubel, F.M. et al. eds., Short Protocols In Molecular Biology (4th Ed. 1999) John Wiley & Sons, NY. (ISBN 0-471-32938-X).

Freshney, R.I. Culture of Animal Cells (1987) Alan R. Liss, Inc.

Old, R.W. & S.B. Primrose, Principles of Gene Manipulation: An Introduction To Genetic Engineering (3d Ed. 1985) Blackwell Scientific Publications, Boston. Studies in Microbiology; V.2:409 pp. (ISBN 0-632-01318-4).

Sambrook, J. et al. eds., Molecular Cloning: A Laboratory Manual (2d Ed. 1989) Cold Spring Harbor Laboratory Press, NY. Vols. 1-3. (ISBN 0-87969-309-6).

Winnacker, E.L. From Genes To Clones: Introduction To Gene Technology (1987) VCH Publishers, NY (translated by Horst Ibelgaufts). 634 pp. (ISBN 0-89573-614-4).

The thermostable chimeric nucleic acid polymerases described herein are especially useful for generating a desired target nucleic acid. Thermostable chimeric nucleic acid polymerases of the invention, having at least two enzymatically active domains that are not naturally associated may be utilized under conditions sufficient to allow polymerization of a nascent nucleic acid. Generally, this method includes any method of nucleic acid generation, replication, amplification, transcription, or reverse transcription known in the art that utilizes a conventional nucleic acid polymerase, wherein the nucleic acid polymerase is substituted or combined with a chimeric nucleic acid polymerase of the present invention. Preferably the method of amplification is polymerase chain reaction, utilizing a thermostable chimeric nucleic acid polymerase. PCR is

described herein as an exemplary protocol capable of utilizing the compositions and methods of the present invention without limitation. Persons skilled in the art will understand that the present invention has utility in other processes requiring the polymerization of nucleic acid (e.g., RT-PCR).

PCR is a technique well known in the art. PCR is used to amplify nucleic acids by subjecting a reaction mixture to cycles of: (i) nucleic acid denaturation, (ii) oligonucleotide primer annealization, and (iii) nucleic acid polymerization. Preferred reaction conditions for amplification comprise thermocycling, i.e., alternating the temperature of the reaction mixture to facilitate each of the steps of the PCR cycle. PCR is typically extended through multiple cycles of denaturation, annealization and replication, augmented (optionally and preferably) with an initial prolonged denaturation step and a final prolonged extension (polymerization) step. To perform the repetitive steps of thermocycling, it is preferable to employ an enzyme that is capable of tolerating exposure to relatively high temperature without a subsequent significant loss in enzyme activity; i.e., a thermostable enzyme. The use of a thermostable enzyme for PCR protocols permits the repetitive steps of increasing and decreasing reaction temperatures without the need to supplement, or otherwise add, enzyme after each successive high temperature step of the PCR program cycle.

Also included in the invention is a kit that includes a thermostable chimeric nucleic acid polymerase and one or more additional reagents suitable for nucleic acid polymerization reactions. Such components may include, but are not limited to: one or more additional enzymes, one or more oligonucleotide primers, a nucleic acid template, any one or more nucleotide bases, an appropriate buffering agent, a salt, or other additives useful in nucleic acid polymerization.

Additional enzymes of the kit include any enzyme that may be used in combination with the thermostable chimeric nucleic acid polymerase of the invention. For example, multiple-polymerase kits are known in the art. Numerous polymerases are known and commercially available to persons skilled in the art, and include DNA polymerases, RNA polymerases, and reverse transcriptases (commercial suppliers include: Roche Diagnostics., Indianapolis, IN; Life Technologies, Inc., Rockville, MD; New England Biolabs, Inc., Beverly, MA; Perkin Elmer Corp., Norwalk, CT; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ; Qiagen, Inc., Valencia, CA; Stratagene, La Jolla, CA).

Oligonucleotide primers useful in the present invention may be any oligonucleotide of two or more nucleotides in length. Preferably, PCR primers are about 15 to about 30 bases in length and are not palindromic (self-complementary) or complementary to other primers that may be used in the reaction mixture. Primers may be, but are not limited to, random primers, homopolymers, or primers specific to a target oligonucleotide template

(e.g., a sequence specific primer). Oligonucleotide primers are oligonucleotides used to hybridize to a region of a target nucleic acid to facilitate the polymerization of a complementary nucleic acid. In PCR protocols, primers serve to facilitate polymerization of a first nucleic acid molecule complementary to a portion of an oligonucleotide template, and also to facilitate replication of the oligonucleotide. Any primer may be synthesized by a practitioner of ordinary skill in the art or may be ordered and purchased from any of a number of commercial venders (e.g., from Roche Diagnostics, Indianapolis, IN; Life Technologies, Inc., Rockville, MD ; New England Biolabs, Inc., Beverly, MA; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). It will be understood that a vast array of primers may be useful in the present invention, including those not specifically disclosed herein, without departing from the scope or preferred embodiments thereof.

A nucleic acid template is defined as any polynucleotide molecule used to provide a nucleic acid sequence from which a polynucleotide complementary to the template may be generated. The synthesis of DNA from a DNA template may be accomplished according to the invention by utilizing a thermostable chimeric DNA polymerase. The synthesis of RNA from a DNA template may be accomplished according to the invention by utilizing a thermostable chimeric RNA polymerase. The synthesis of DNA from an RNA template may be accomplished according to the invention by utilizing a thermostable chimeric nucleic acid polymerase that exhibits reverse transcriptase activity.

Nucleotide bases useful in the present invention may be any nucleotide useful in the polymerization of a nucleic acid. Nucleotides may be naturally occurring, unusual, modified, derivative, or artificial. Nucleotides may be unlabeled, or detectably labeled by methods known in the art (e.g., using radioisotopes, vitamins, fluorescent or chemiluminescent moieties, digoxigenin). Preferably the nucleotides are deoxynucleoside triphosphates, dNTPs (e.g., dATP, dCTP, dGTP, dTTP, dITP, dUTP, α-thioSONDZEICHEN-dNTPs, biotin-dUTP, fluorescein-dUTP, digoxigenin-dUTP, 7-deaza-dGTP). dNTPs are also well known in the art and are commercially available (e.g., from Roche Diagnostics, Indianapolis, IN; New England Biolabs, Inc., Beverly, MA; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ).

Buffering agents and salts useful in the present invention provide appropriate stable pH and ionic conditions for nucleic acid synthesis. A wide variety of buffers and salt solutions and modified buffers are known in the art that may be useful in the present invention, including agents not specifically disclosed herein. Preferred buffering agents include, but are not limited to, TRIS, TRICINE, BIS-TRICINE, HEPES, MOPS, TES, TAPS, PIPES, CAPS. Preferred salt solutions include, but are not limited to solutions of; potassium chloride, potassium acetate, potassium sulfate, ammonium sulfate, ammonium chloride, ammonium acetate, magnesium chloride, magnesium acetate, magnesium

sulfate, manganese acetate, sodium chloride, sodium acetate, lithium chloride, and lithium acetate.

Other additives capable of facilitating nucleic acid generation and amplification, other than those disclosed for the first time by this invention, are known in the art. In accordance with the compositions and methods of this invention, one or more of these additives may be incorporated in a DNA/RNA polymerization kit according to the present invention to optimize the generation and replication of polynucleotides. Additives may be organic or inorganic compounds. Agents useful in the present invention include, but are not limited to, polypeptides such as phosphatase, human serum albumin, bovine serum albumin (BSA), ovalbumin, albumax, casein, gelatin, collagen, globulin, lysozyme, transferrin, α-lactalbumin, β-lactoglobulin, phosphorylase b, myosin, actin, β-galactosidase, lectins, *E. coli* single-stranded binding (SSB) protein, phage T4 gene 32 protein, and the like, or fragments or derivatives thereof. Examples of nonpolypeptide additives include, but are not limited to; homopolymeric nucleic acid, heteropolymeric nucleic acid, tRNA, rRNA, sulfur-containing compounds, acetate-containing compounds, dimethylsulfoxide (DMSO), glycerol, formamide, betain, tetramethylammonium chloride (TMAC), polyethylene glycol (PEG), Tween 20, NP 40, ectoine, and polyoles.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the compositions and methods of the invention described herein are obvious and may be made without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLE 1: Construction of a Thermostable Chimeric DNA Polymerase Gene

Chimeric thermostable DNA polymerase constructs containing enzymatically active domains from different (source) thermostable DNA polymerases were generated using recombinant DNA techniques. The 3'-5' exonuclease domain of various thermostable polymerases were recombinantly linked to the 5'-3' polymerase domain of *Taq* polymerase or *Tth* polymerase. The particularly preferred enzymatic domains and domain borders, described herein in detail, were selected and tested as preferred embodiments, and are not to be considered limiting in scope of the thermostable chimeric nucleic acid polymerase of the invention, or the enzymatically active domains useful therein.

Appropriate microbial strains or genomic DNA preparations, from which the enzymatically active domains used in the construction of chimeric nucleic acid polymerase were isolated, were purchased from commercial suppliers, e.g., from DSMZ GmbH

(Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), Braunschweig, Germany. Specifically chosen strains included *Thermus aquaticus* (order # DSM 625), *Thermus thermophilus* (order # DSM 579), *Pyrococcus furiosus* (order # DSM 3638), *Pyrococcus woesei* (order # DSM 3773), *Pyrococcus horikoshii* (order # DSM 3638), *Sulfolobus solfataricus* (order # DSM 5833), and *Thermococcus pacificus* (order # DSM 10394). A multiplicity of genomic DNA extraction, purification, and isolation techniques useful to obtain the desired enzymatically active domains are well known in the art.

Modified PCR amplification techniques and/or cloning procedures such as restriction digestion and ligation using appropriate enzymes were used to obtain the chimeric DNA polymerase constructs. Primers appropriate to amplify polynucleotides encoding particular enzymatic domains from the source thermostable DNA polymerases were synthesized according to the nucleotide sequences of the source thermostable DNA polymerase. DNA sequences of the source thermostable DNA polymerases are published in GenBank. The synthesis of oligonucleotide primers is well known to practitioners in the art, and may also be ordered from commercial oligonucleotide suppliers (e.g., Life Technologies, Gaithersburg, MD).

PCR primers were of special design. The primers contained a nucleotide sequence complementary to the terminal region of a particular enzymatic domain of interest within a source DNA polymerase. The primers also contained a noncomplementary nucleotide sequence region as well to provide; i) an appropriate restriction enzyme site, to facilitate genetic manipulation (e.g., vector insertion), or ii) sequence information (e.g., complementarity), to facilitate fusion to a second, non-naturally associated enzymatic domain. For example, primers designed to facilitate fusion of a 3'-5' exonuclease domain to a 5'-3' polymerase domain contained a sequence, one half of which was complementary to a terminal region of the 3'-5' exonuclease domain of interest (e.g., residues 388-396 of *Pho* polymerase) and one half of which was complementary to a terminal region of the 5'-3' polymerase domain (e.g., residues 281-288 of *Taq* polymerase).

As an initial step, various enzymatic domains were amplified by PCR. The PCR reaction mixture contained: 2.5 units of *Taq* polymerase (Qiagen, Valencia, CA) and 0.1 to 0.2 units of *Pfu* polymerase (Stratagene, La Jolla, CA); an appropriate amount of the specially designed primers, as described above (0.2 to 1.0 μM); genomic DNA isolated from the appropriate microorganism containing the source thermostable polymerase; and 200 μM of each dNTP in a 1x PCR buffer (Qiagen, Valencia, CA). A 3-step PCR cycling program was run, consisting of an initial denaturation step at 94° C, an annealing step and an extension step. The PCR ran for 25-35 cycles, depending upon the desired amount of product. The size of the PCR product was checked by agarose gel electrophoresis

against an appropriate DNA size marker. The correctly sized PCR product was gel-purified using the QIAquick™ Gel Extraction Kit (Qiagen, Valencia, CA).

Once isolation and amplification of the polynucleotides encoding the enzymatic domains chosen for chimeric polymerase construction were obtained, the component enzymatic domains were combined, in equivalent concentrations, in a composite PCR reaction, together with 2-5 units of *Pfu* polymerase (Stratagene, La Jolla, CA), and 200 μM of each dNTP in 1x PCR buffer (Qiagen, Valencia, CA). This PCR mixture did not contain any primer oligonucleotides. This reaction mixture was subjected to 10 to 15 PCR cycles.

During the composite PCR , the single strand polynucleotides encoding each of the enzymatically active domains hybridize at their respective terminal regions of complementarity (due to the specially designed primers as described above). The hybridized single strand polynucleotides encoding each of the enzymatically active domains form a single composite polynucleotide template, thus serving as primers for each other. *Pfu* polymerase extends the 3' terminal end of each of the enzymatically active domains, creating a single polynucleotide containing the chimeric DNA polymerase gene construct.

After the initial 10 to 15 cycles of chimeric DNA polymerase gene construction, oligonucleotide primers, appropriate to amplify the full-length chimeric DNA polymerase gene, were added to the PCR mixture. The PCR ran for 20-30 additional cycles, depending upon the desired amount of chimeric DNA polymerase PCR product. The size of the PCR product was checked by agarose gel electrophoresis and the correctly sized PCR product was gel-purified as described above.

The purified chimeric DNA polymerase gene was then subjected to restriction digestion with the appropriate restriction enzyme to cut the polynucleotide at restriction sites located at the terminal ends of the chimeric DNA polymerase gene. These sites were originally generated by the specially designed primers described above.

EXAMPLE 1.1: Construction of a *Pho*/*Taq* Thermostable Chimeric DNA Polymerase Gene

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Pho* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain and the nonfunctional 3'-5' exonuclease domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-396 (SEQ ID NO:3) of *Pho* DNA polymerase following the procedures detailed in

Example 1 above, producir g a polynucleotide that encodes a novel *Pho/Taq* thermostable chimeric DNA polymerase (SEQ ID NO:8).

EXAMPLE 1.2: Construction of a *Pwo/Taq* Thermostable Chimeric DNA Polymerase Gene

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Pwo* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-396 (SEQ ID NO:4) of *Pwo* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Pwo/Taq* thermostable chimeric DNA polymerase (SEQ ID NO:9).

EXAMPLE 1.3: Construction of a *Sso/Taq* Thermostable Chimeric DNA Polymerase Gene

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Sso* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 281-832 (SEQ ID NO:1) of *Taq* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-508 (SEQ ID NO:6) of *Sso* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Sso/Taq* thermostable chimeric DNA polymerase (SEQ ID NO:10).

This chimeric construct, possessing a smaller *Taq* 5'-3' polymerase domain than that used in Examples 1.1 and 1.2, also demonstrates that specifically determined domain borders of an enzymatic domain are not essential to the invention. What is essential for the domain is that it retain its definitive enzymatic activity.

EXAMPLE 1.4: Construction of a *Tpac/Taq* Chimeric DNA Polymerase Gene

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Tpac* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-395 (SEQ ID NO:16) of *Tpac* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Tpac/Taq* chimeric DNA polymerase (SEQ ID NO:17).

EXAMPLE 1.5: Construction of Variant Thermostable Chimeric DNA Polymerase Genes

To further demonstrate that a thermostable chimeric nucleic acid polymerase may be generated using an enzymatically active domain of varying domain borders (provided the enzymatic activity of the domain is retained), a *Pwo*/*Taq* chimeric DNA polymerase variant of the thermostable chimeric polymerase generated in Example 1.2 was constructed. This variant construct comprised a polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA polymerase recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-421 (SEQ ID NO:5) of *Pwo* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a second novel *Pwo*/*Taq* thermostable chimeric DNA polymerase (SEQ ID NO:11).

EXAMPLE 1.6: Construction of a *Pho*/*Tth* Thermostable Chimeric DNA Polymerase Gene

To demonstrate that a thermostable chimeric nucleic acid polymerase may be generated using an enzymatically active polymerase domain other than that of *Taq* polymerase, a polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Pho* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Tth* DNA polymerase. A polynucleotide encoding amino acids 273-834 (SEQ ID NO:2) of *Tth* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-396 (SEQ ID NO:3) of *Pho* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Pho*/*Tth* thermostable chimeric DNA polymerase (SEQ ID NO:12).

This chimeric construct, possessing a *Tth* 5'-3' polymerase domain that is also capable of reverse transcription activity, also demonstrates a thermostable chimeric nucleic acid polymerase of the present invention useful for RT-PCR protocols.

EXAMPLE 1.7: Construction of a Thermostable Chimeric DNA Polymerase Gene Encoding More Than Two Enzymatically Active Domains

The chimeric nucleic acid polymerase gene of the invention may encode two or more enzymatically active domains, of which two more domains are non-naturally occurring. In addition the enzymatically active domains may be derived from any polypeptide source naturally occurring or synthetically produced.

For example, the practitioner may wish to construct a thermostable chimeric nucleic acid polymerase possessing both the 5'-3' polymerase domain and the 5'-3' exonuclease domain of *Taq* polymerase, as well as the 3'-5' exonuclease domain of another

polymerase (e.g., *Pho* polymerase). In this instance, a polynucleotide encoding the 5'-3' exonuclease domain of *Taq* polymerase (known to be contained within amino acids 1-291 of *Taq* polymerase) would be recombinantly linked to 5' end of a polynucleotide encoding the 3'-5' exonuclease domain of *Pho* polymerase (e.g., SEQ ID NO: 3) and the 5'-3' polymerase domain of *Taq* DNA polymerase (e.g., SEQ ID NOs: 1 or 7), which was earlier demonstrated in Examples 1.1 and 1.5.

EXAMPLE 2: Construction of a Thermostable Chimeric DNA Polymerase Vector

The isolated chimeric DNA polymerase genes of Examples 1.1 through 1.6 were each ligated into a vector, linearized using the appropriate restriction enzyme. Ligation was performed overnight at 16° C using T4 DNA ligase and an appropriate buffer (Life Technologies, Gaithersburg, MD) in a final volume of 20 µl.

EXAMPLE 3: Construction of a Thermostable Chimeric DNA Polymerase Host Cell

The ligated recombinant vectors of Example 2 were used to transform calcium-competent M15[pRep4] cells (Qiagen, Valencia, CA ) or DH5SONDZEICHENα competent cells. Aliquots of the transformation mixture were spread onto agar plates containing ampicillin and kanamycin (for M15[pRep4] cells), or ampicillin only (for DH5α competent cells), and incubated overnight at 37° C.

Colonies of successfully transformed cells were transferred to LB media containing the appropriate antibiotic selection, and incubated overnight. Plasmid isolation preparations were performed using QIAprep™ Spin Kit or Plasmid Midi Kit (both from Qiagen, Valencia, CA). Presence of the chimeric DNA polymerase gene was verified by restriction digest analysis and the chimeric DNA polymerase gene sequenced by techniques well known in the art.

The chimeric DNA polymerase genes were cloned into either pQE-30 or pQE-31 expression vectors (Qiagen, Valencia, CA) containing a six-histidine tag sequence preceding the respective DNA polymerase sequence.

EXAMPLE 4: Expression and Purification of a Thermostable Chimeric DNA Polymerase

Thermostable chimeric DNA polymerase gene expression of the successfully transformed host cells from Example 3, was induced by IPTG. Harvested cells were lysed by sonification and lysozyme treatment or a simple heat treatment. Chimeric His-tagged protein was purified in batch format using Ni-NTA agarose (Qiagen, Valencia, CA) following standard protocol procedures.

Eluates were ultrafiltrated using Nanosep[SONDZEICHEN®] ultrafiltration units (Pall Deutschland GmbH Holding, Dreieich, Germany). Alternatively, heat treated cleared lysate was centrifuged through Ultrafree filterunits 300.000 (Sigma, Deisenhofen, Germany), to remove contaminating nucleic acids, and was subsequently concentrated using Nanosep[SONDZEICHEN®] or Microsep[SONDZEICHEN®] ultrafiltration units (Pall Deutschland GmbH Holding, Dreieich, Germany).

Concentrated samples were mixed with a storage buffer containing 20 mM TrisHCl (pH 8.0 at 20º C), 100 mM KCl, 1 mM EDTA, 0.5% (v/v) Nonidet P-40 substitute, 0.5% (v/v) Tween 20 and 50% (v/v) glycerol. Chimeric polymerase preparations were stored at -20º C. In some cases, the cleared lysate of the polymerase preparation was directly used for subsequent analysis; chimeric polymerase preparations were then stored at +4ºC.

EXAMPLE 5: 5'-3' Polymerase Activity of Thermostable Chimeric DNA Polymerases

To demonstrate the polymerase activity of thermostable chimeric DNA polymerases produced from Example 4, an assay for measuring primer extension activity was performed. This assay is based on the difference in mobility of single- versus double-strand DNA molecules on an agarose gel in the presence of a DNA intercalating dye. Annealing of a primer to a single-stranded DNA molecule creates a priming site for a DNA polymerase. The primer is then extended by the polymerase, converting the single-strand DNA into double-strand molecules. The extension rate is dependent upon the polymerase used. The final amount of DNA extension (i.e., polymerization) is dependent on the amount of polymerase provided, the extension rate of the polymerase, and the length of time the reaction is allowed to proceed.

All polymerization reaction mixtures contained 50 ng M13mp18 DNA (20 fmol; 7250 nt), 0.1 µM 30-mer oligonucleotide primer 5'-TTTCCCAGTCACGACGTTGTAAAACGACGG-3' (SEQ ID NO: 13), and 50 µM of each dNTP in 10 µl of 10 mM Tris HCl.

Polymerization reactions containing *Taq* DNA polymerase and a thermostable chimeric DNA polymerase were performed in 1x PCR buffer (Qiagen, Valencia, CA).

*Taq* DNA polymerase was used for external standard reactions (0.05, 0.03, 0.01 units) in order to determine polymerase activity of the thermostable chimeric DNA polymerases. DNA polymerases were diluted in the reaction buffer containing 1 µg/ml bovine serum albumin (BSA) to compensate for possible protein interactions with the surface of the polypropylene tube.

The assay was performed in a MJ Research PTC-200 Thermocycler (Biozym, Hess. Oldendorf, Germany) or a Biometra Uno II Thermocycler (Biometra, Göttingen,

Germany). The thermal program consisted of a 10 sec. denaturation step 94º C; a 30 sec. annealing step at 55º C; and a 3 min. polymerization step at 72º C. Heating of the reaction mixture to 94º C was done to destroy possible secondary structures of the single-stranded M13 DNA and to facilitate specific primer annealing during the lowering of reaction temperature to 55º C.

Results of primer extension reactions at 72º C were reproducible. After completing the reaction, reaction products were mixed with 1 µl gel loading solution (50% Glycerol, 1x TAE buffer, 0.02 mg/ml Bromphenol blue) and loaded on a 1% agarose gel containing 0.5 µg/ml ethidium bromide. The gel was run at 80 mA for 15 min in 1x TAE buffer. These conditions facilitated discrimination between extended- (ds) and non-extended (ss) M13 DNA fragments. The results, as represented in Fig 1, illustrate the polymerase activity of the thermostable chimeric polymerase is comparable to that of wild type *Taq* polymerase.

EXAMPLE 6: Thermostability of Chimeric DNA Polymerases

The primer extension assay described in Example 5 was also used to measure the resilience of chimeric DNA polymerases to thermal degradation (i.e., thermostability). Heat-treatment of chimeric DNA polymerases (0.2 units) consisted of incubation of the enzyme for 0, 10, 15, 30, 60, 90 and 120 min at 90°C, followed by primer extension at 72°C. Polymerase activity of heat-treated chimeric polymerase was compared to untreated chimeric DNA polymerase based on the amount of polymerized (i.e., double strand) M13 DNA. The same assay was performed, under identical reaction conditions, on identical amounts of *Taq* DNA polymerase, as a standard. A control consisted of a polymerase reaction mixture, without any DNA polymerase. After completing the reaction, reaction products were mixed with 1 µl gel loading solution (50% Glycerol, 1x TAE buffer, 0.02 mg/ml Bromphenol blue), and loaded on a 1% agarose gel containing 0.5 µg/ml ethidium bromide. The gel was run at 80 mA for 15 min in 1x TAE buffer. The results, presented in Fig 2 and quantified in Table 2 below, are representative of the thermostability assay.

**Table 2. Thermostability of chimeric polymerase compared to *Taq* polymerase**

| Incubation at 90ºC (min.) | *Pho/Taq* Chimeric Polymerase % Activity | *Taq* Polymerase % Activity |
|---|---|---|
| 0 | 100 | 100 |
| 10 | 84 | 99 |
| 15 | 84 | 89 |
| 30 | 82 | 74 |
| 60 | 66 | 69 |
| 90 | 53 | 31* |
| 120 | 45 | 43 |

* single non-reproducible data; value expected to be higher

Figure 2 and Table 2 confirm the thermostability of the chimeric polymerase of the present invention. Table 2 illustrates that although the activity of the chimeric DNA polymerase shows an initial drop in activity (within the first 10 min at 90ºC) greater than that of *Taq* DNA polymerase, the overall thermostability is comparable to *Taq* DNA polymerase. Chimeric DNA polymerase of the invention displays the same half life at 90ºC as *Taq* DNA polymerase (approximately 90 min).

The thermostability assay was also performed under extreme temperature conditions. The primer extension assay was run after heat-treatment at 95°C for 0, 3, 5, and 10 min. The results, quantified in Table 3 below, are representative of the 95°C thermostability assay, and further confirm that the chimeric DNA polymerase of the present invention is highly thermostable.

**Table 3. Thermostability of chimeric polymerase**

| Incubation at 95ºC (min.) | *Pho/Taq* Chimeric Polymerase % Activity |
|---|---|
| 0 | 100 |
| 3 | 100 |
| 5 | 86 |
| 10 | 86 |

These results confirm the thermostability of the chimeric DNA polymerase of the present invention, making it useful for *in vitro* reactions under heat denaturing conditions such as PCR, without requiring successive addition of enzyme at each cycle of the PCR program.

EXAMPLE 7: 3'-5' Exonuclease Activity of Thermostable Chimeric DNA Polymerases

Fidelity of DNA replication is based on a two step process: misinsertion and misextension. In PCR, if the DNA polymerase inserts an incorrect nucleotide, and the

resulting 3'-mismatched terminus of the growing DNA chain is not extended, the truncated primer extension product cannot be amplified during subsequent PCR cycles since the downstream primer binding site is missing. Additionally, mismatched termini are less efficiently extended than DNA ends harboring the complementary base. DNA polymerases possessing an enzymatically active 3'-5' exonuclease domain are capable of removing a misincorporated nucleotide, thus increasing fidelity of the PCR product and increasing primer extension efficiency.

A PCR and restriction endonuclease digestion assay, developed to assess the ability of thermostable DNA polymerases to remove mismatched primer termini by 3'-5' exonuclease activity, was performed using the protocol disclosed in U.S. Patent No. 5,491,086 (incorporated by reference). Wild type primers, perfectly matching the *Bam*HI restriction enzyme recognition sequence in the *Taq* polymerase gene, and mutant primers, possessing a 3'-mismatch (employing every possible combination) to the first nucleotide of the *Bam*HI restriction enzyme recognition sequence, were used in side-by-side PCR trials.

Wild type primers to 5'-GCACCCCGCTTGGGCAGAG-3' (SEQ ID NO:14) and 5'-TCCCGCCCCTCCTGGAAGAC-3' (SEQ ID NO:15) yield a 151 bp PCR product that becomes digested upon incubation with *Bam*HI restriction enzyme, generating a 132 bp and 19 bp fragment.

Three forward primers containing a single 3'-mismatched nucleotide representing a C:A, C:T, and C:C mismatch to SEQ ID NO:14 were used as mutant primers. Any extension product from these mutant primers would corrupt the *Bam*HI restriction site, rendering the resulting PCR products unaffected by *Bam*HI digestion, thus leaving the 151 bp PCR product intact. The presence of an enzymatically active 3'-5' exonuclease domain, would correct the 3'-mismatched nucleotide of the mutant primer, however, thus restoring the *Bam*HI restriction site, rendering the PCR product susceptible to *Bam*HI digestion, thus producing the 132 bp and 19 bp digestion fragments.

Using this PCR fidelity assay, the chimeric thermostable DNA polymerase was tested for the ability to correct a 3'-primer mismatch during PCR. Chimeric polymerase trials were run in parallel with wild type *Taq* DNA polymerase and *Pfu* DNA polymerase I. The *Taq* DNA polymerase trials served as a negative control, representing a DNA polymerase possessing an enzymatically inactive 3'-5' exonuclease domain (i.e., proofreading capability). The *Pfu* DNA polymerase I trials served as a positive control, representing a thermostable DNA polymerase possessing an enzymatically active 3'-5' exonuclease domain.

PCR mixtures comprised 20 ng plasmid pQE-31 containing the (target) *Taq* polymerase gene sequence; 0.5 units of the test DNA polymerase; 0.4 µM of the

appropriate trial primers (wild type vs. mutant primers); 200 µM of each dNTP; 1x Qiagen PCR buffer (Qiagen, Valencia, CA) or 1x Pfu reaction buffer (Stratagene, La Jolla, CA) and 1.5 mM $MgCl_2$ in a final reaction volume of 50 µl.

PCR was performed using a MJ Research PTC-200 Thermocycler (Biozym, Hess. Oldendorf, Germany) or a Biometra Uno II Thermocycler (Biometra, Göttingen, Germany). The PCR program consisted of an initial 1 min template denaturation step at 94º C followed by 40 cycles of a 30 sec. denaturation step 94º C; a 30 sec. annealing step at 62º C; and a 1 min. polymerization step at 72º C for 1 min. The PCR concluded with a final prolonged extension step for 2 min. at 72º C.

PCR products were analyzed on a 2% agarose gel by gel electrophoresis (approximately 35 min. at 85 volts) in 1x TAE electrophoresis buffer and Ethidium bromide. PCR products were visualized using UV irradiation, and quantified using the 200 bp DNA fragment of the Low DNA MassSONDZEICHEN™ Ladder (Life Technologies, Gaithersburg, MD, USA) as standard by gel densitometry. PCR products were purified using QIAquick™ PCR Purification Kit (Qiagen, Valencia, CA).

Identical amounts of PCR product were digested in the same final reaction volume using 1 unit *Bam*HI (Life Technologies, Gaithersburg, MD, USA) per 100 ng PCR product and corresponding reaction buffer. Restriction digest was performed for 90 min. at 37º C. Digestion products were analyzed on a 4% Metaphor$^{SONDZEICHEN®}$ agarose gel (Biozym, Hess. Oldendorf, Germany). Fig. 3. is representative of the results of the 3'-5' exonuclease activity assay.

Fig 3(A) illustrates the PCR product of the three nucleic acid polymerases (*Taq* polymerase, *Pfu* polymerase, and the thermostable chimeric polymerase) using wild type primers. Alternating lanes represent undigested PCR product and PCR product subjected to *Bam*HI digestion. Undigested product shows the intact 151 bp PCR product. Digestion treated product shows the 132 bp digestion fragment.

Fig 3(B) illustrates the PCR product of the three polymerases (*Taq* polymerase, *Pfu* polymerase, and the thermostable chimeric polymerase) using mutant primers. Once again, alternating lanes represent undigested PCR product and PCR product subjected to *Bam*HI digestion. *Taq* polymerase PCR product was unaffected by *Bam*HI digestion (lanes 3 and 5), due to the lack of a *Bam*HI site resulting for normal extension of the mutant primer. *Pfu* polymerase PCR product was effectively digested by *Bam*HI (lanes 7 and 9), producing the expected 132 bp digestion fragment. These results are indicative of the proofreading ability (i.e., 3'-5' exonuclease activity) of *Pfu* polymerase, which corrected the nucleotide mismatch of the mutant primer, thus restoring the *Bam*HI site of the template DNA.

The thermostable chimeric polymerase PCR product displayed results similar to the *Pfu* polymerase PCR product. The chimeric polymerase PCR product was also effectively digested by *Bam*HI (lanes 11 and 13), producing the expected 132 bp digestion fragment and indicative of polymerase proofreading ability. These results confirm that the thermostable chimeric polymerase, which possesses the 5'-3' polymerase domain of *Taq* polymerase, also possesses an enzymatically active 3'-5' exonuclease domain not naturally occurring in *Taq* polymerase.

EXAMPLE 8: PCR efficiency of Thermostable Chimeric DNA Polymerases

PCR efficiency of a DNA polymerase can be described as the combined effect of primer extension activity and processivity of the enzyme. PCR efficiency of the thermostable chimeric DNA polymerase was tested in comparison with *Taq* DNA polymerase, known to possess a higher PCR efficiency than common proofreading polymerases, and *Pfu* DNA polymerase (both serving as controls).

One unit of the respective polymerase was used to amplify a 750 bp large product from human genomic DNA using a thermocycling profile with varying primer extension times at 72ºC. Limiting primer extension time was used to measure polymerase efficiency in PCR, using the same amount of enzyme activity in the assay. *Taq* DNA polymerase was assayed in its optimized PCR buffer (Qiagen, Valencia, CA), a *Pho/Taq* thermostable chimeric DNA polymerase was used in a 1x buffer consisting of 50 mM TrisHCl (pH 8.9 at room temperature), 10 mM $(NH_4)_2SO_4$, and *Pfu* DNA polymerase was used in the reaction buffer supplied with the enzyme (Stratagene, La Jolla, CA). All reactions contained 1 unit of enzyme, 0.4 µM of each primer, 200 µM of each dNTP, and a final $MgCl_2$ concentration of 1.5 mM (*Taq* polymerase, chimeric DNA polymerase) or 2.0 mM (*Pfu* polymerase).

Thermocycling was performed in a Biometra Uno thermocycler using the following cycling conditions: initial denaturation at 94ºC for 3 min followed by a denaturation step at 94ºC for 30 sec, an annealing step at 60ºC for 30 sec, and a primer extension step at 72ºC for 1 min, 30 sec, 10 sec or 5 sec. The reaction proceeded for 34 cycles, and concluded with a final extension step at 72ºC for 10 min.

The results are depicted in Figure 4. *Taq* DNA polymerase **(A)** shows a high PCR efficiency even when primer extension time is as low as 5 sec. The thermostable chimeric DNA polymerase **(B)** shows a higher PCR efficiency than *Taq* polymerase at extension times of 1 min and 30 sec, but a slightly lower efficiency than *Taq* polymerase at 5 sec extension time. *Pfu* DNA polymerase I **(C)** generates a visible PCR product only when using the 1 min extension time.

These results indicate that the overall processivity of the chimeric polymerase is comparable to that of *Taq* DNA polymerase, and is dramatically better than *Pfu* DNA polymerase I. The thermostable chimeric polymerase of the present invention performs as well as *Taq* DNA polymerase (the standard enzyme of PCR protocols) , and outperforms *Pfu* DNA polymerase I (the standard enzyme for high fidelity PCR protocols). In addition, the thermostable chimeric polymerase of the present invention combines the beneficial features of each of the standard enzymes for PCR protocols formerly not obtained with either *Taq* DNA polymerase or proofreading polymerases: removal of misincorporated nucleotides required for high fidelity PCR, and high PCR efficiency.

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Each of the publications mentioned herein is incorporated by reference.

We claim:

1. A chimeric nucleic acid polymerase comprising at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, and said chimeric nucleic acid polymerase is thermostable.

2. The chimeric nucleic acid polymerase of claim 1, wherein said domains are derived from enzymes of different species.

3. The polymerase of claim 1, wherein said domains are selected from the group consisting of: 5'-3' exonuclease domain, 3'-5' exonuclease domain, and 5'-3' polymerase domain.

4. The polymerase of claim 3, wherein at least one of said domains is a 5'-3' polymerase domain.

5. The polymerase of claim 3, wherein at least one of said domains is a 3'-5' exonuclease domain.

6. The polymerase of claim 1, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by a thermophilic organism.

7. The polymerase of claim 1, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by an organism of a genus selected from the group of genera consisting of: Thermus, Thermococcus, Thermotoga, Pyrococcus, Pyrodictium, Bacillus, Sulfolobus, and Methanobacterium.

8. The polymerase of claim 1, wherein at least one of said enzymatically active domains is isolated from a polymerase selected from the group of DNA polymerases consisting of:
*Tac* polymerase; *Taq* polymerase; *Tba* polymerase; *Tbr* polymerase; *Tfi* polymerase;
*Tfl* polymerase; *Tfu* polymerase; *Tih* polymerase; *Tli* polymerase; *Tpac* polymerase;
*Tru* polymerase; *Tth* polymerase; *Pab* polymerase; *Pfu* polymerase; *Phe* polymerase;
*Pho* polymerase; *Pko* polymerase; *Poc* polymerase; *Psp* polymerase; *Pwo* polymerase;
*Tma* polymerase; *Tne* polymerase; *Bst* polymerase; *Sac* polymerase; *Sso* polymerase;

*Mth* polymerase; Kod polymerase; ES4 polymerase;
and mutants, variants, and derivatives thereof.

9. The polymerase of claim 1, wherein said polymerase comprises a 3'-5' exonuclease domain and a 5'-3' polymerase domain.

10. The polymerase of claim 9 wherein said 5'-3' polymerase domain is selected from the group consisting of; a 5'-3' polymerase domain of *Taq* DNA polymerase; a 5'-3' polymerase domain of *Tth* DNA polymerase, and mutants, variants, or derivatives thereof.

11. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a Stoffel fragment of *Taq* DNA polymerase or a mutant, variant, or derivative thereof.

12. The polymerase of claim 10 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1).

13. The polymerase of claim 10 wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7).

14. The polymerase of claim 10 wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2).

15. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or a mutant, variant, or derivative thereof.

16. The polymerase of claim 15 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).

17. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or a mutant, variant, or derivative thereof.

18. The polymerase of claim 17 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).

19. The polymerase of claim 17 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).

20. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase, mutants, variants, or derivatives thereof.

21. The polymerase of claim 20 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).

22. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase, mutants, variants, or derivatives thereof.

23. The polymerase of claim 22 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).

24. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.

25. The polymerase of claim 24 wherein, said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).

26. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or mutant, variant, or derivative thereof.

27. The polymerase of claim 26 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).

28. The polymerase of claim 26 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).

29. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase or mutant, variant, or derivative thereof.

30. The polymerase of claim 29 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).

31. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase or mutant, variant, or derivative thereof.

32. The polymerase of claim 31 wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).

33. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Tth* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.

34. The polymerase of claim 33 wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).

35. The polymerase of claim 9 comprising a polypeptide selected from the group consisting of: SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:17; and mutants, variants, or derivatives thereof.

36. An isolated polynucleotide encoding a thermostable chimeric nucleic acid polymerase comprising at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated.

37. The isolated polynucleotide of claim 36, wherein said domains are derived from different species.

38. The isolated polynucleotide of claim 36, wherein said domains are selected from the group consisting of: 5'-3' exonuclease domain, 3'-5' exonuclease domain, and 5'-3' polymerase domain.

39. The isolated polynucleotide of claim 36, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by a thermophilic organism.

40. The isolated polynucleotide of claim 36, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by an organism of a genus selected from the group of genera consisting of: Thermus, Thermococcus, Thermotoga, Pyrococcus, Pyrodictium, Bacillus, Sulfolobus, and Methanobacterium.

41. The isolated polynucleotide of claim 36, wherein at least one of said enzymatically active domains is isolated from a polymerase selected from the group of DNA polymerases consisting of:
*Tac* polymerase; *Taq* polymerase; *Tba* polymerase; *Tbr* polymerase; *Tfi* polymerase;
*Tfl* polymerase; *Tfu* polymerase; *Tih* polymerase; *Tli* polymerase; *Tpac* polymerase;
*Tru* polymerase; *Tth* polymerase; *Pab* polymerase; *Pfu* polymerase; *Phe* polymerase;
*Pho* polymerase; *Pko* polymerase; *Poc* polymerase; *Psp* polymerase; *Pwo* polymerase;
*Tma* polymerase; *Tne* polymerase; *Bst* polymerase; *Sac* polymerase; *Sso* polymerase;
*Mth* polymerase; Kod polymerase; ES4 polymerase;
and mutants, variants, and derivatives thereof.

42. The isolated polynucleotide of claim 36, wherein said polymerase comprises a 3'-5' exonuclease domain and a 5'-3' polymerase domain.

43. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain is selected from the group consisting of; a 5'-3' polymerase domain of *Taq* DNA polymerase; a 5'-3' polymerase domain of *Tth* DNA polymerase, and mutants, variants, or derivatives thereof.

44. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a Stoffel fragment of *Taq* DNA polymerase or a mutant, variant, or derivative thereof.

45. The isolated polynucleotide of claim 43, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1).

46. The isolated polynucleotide of claim 43, wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7).

47. The isolated polynucleotide of claim 43, wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2).

48. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or a mutant, variant, or derivative thereof.

49. The isolated polynucleotide of claim 48, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).

50. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or a mutant, variant, or derivative thereof.

51. The isolated polynucleotide of claim 50, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).

52. The isolated polynucleotide of claim 50, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).

53. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase, mutants, variants, or derivatives thereof.

54. The isolated polynucleotide of claim 53, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).

55. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase, mutants, variants, or derivatives thereof.

56. The isolated polynucleotide of claim 55, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).

57. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.

58. The isolated polynucleotide of claim 57, wherein, said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).

59. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or mutant, variant, or derivative thereof.

60. The isolated polynucleotide of claim 59, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).

61. The isolated polynucleotide of claim 59, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).

62. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase or mutant, variant, or derivative thereof.

63. The isolated polynucleotide of claim 62, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).

64. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or

derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase or mutant, variant, or derivative thereof.

65. The isolated polynucleotide of claim 64, wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).

66. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Tth* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.

67. The isolated polynucleotide of claim 66, wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).

68. The isolated polynucleotide of claim 42, encoding a polypeptide selected from the group consisting of: SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:17; and mutants, variants, or derivatives thereof.

69. A vector comprising a polynucleotide wherein said polynucleotide is selected from the group of polynucleotides of claims 36 to 68.

70. The vector of claim 69 wherein said vector in an expression vector.

71. A recombinant host cell transformed with a vector, said vector comprising a polynucleotide selected from the group of polynucleotides of claims 36 to 68.

72. A kit for polymerization of nucleic acid comprising; a chimeric nucleic acid polymerase according to any one of claims 1 to 35.

73. The kit of claim 72 further comprising at least one reagent suitable for nucleic acid polymerization.

74. The kit of claim 73 wherein said nucleic acid polymerization is nucleic acid amplification.

75. The kit of claim 73 wherein said reagent is selected from the group consisting of; one or more additional enzymes, one or more oligonucleotide primers, a nucleic acid

template, any one or more nucleotide bases, an appropriate buffering agent, and a salt.

76. The kit of claim 75 wherein said reagent is phosphatase.

77. A process for nucleic acid polymerization, comprising the steps of:

(a) providing a chimeric nucleic acid polymerase according to any one of claims 1 to 35; and

(b) contacting said chimeric polymerase with a nucleic acid template under conditions sufficient to allow polymerization of a nascent nucleic acid complementary to said nucleic acid template.

78. A process for synthesizing a recombinant nucleic acid encoding a thermostable chimeric nucleic acid polymerase having at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, comprising the steps of:

(a) isolating nucleic acid encoding a first enzymatically active domain;

(b) isolating nucleic acid encoding a second enzymatically active domain, wherein said second domain is not naturally associated with said first domain; and

(c) genetically combining said nucleic acid encoding a first enzymatically active domain with said nucleic acid encoding a second enzymatically active domain to form a chimeric nucleic acid encoding a thermostable nucleic acid polymerase, wherein said first domain and said second domain are enzymatically active.

79. The process of claim 78, wherein, at least one of said domains is a 5'-3' polymerase domain.

80. The process of claim 78, wherein, at least one of said domains is a 3'-5' exonuclease domain.

81. A process for producing a thermostable chimeric nucleic acid polymerase having at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, comprising the steps of:

(a) culturing a host cell containing nucleic acid encoding said chimeric polymerase under conditions suitable for expression of said chimeric polymerase; and

isolating said chimeric polymerase expressed from said cell culture.

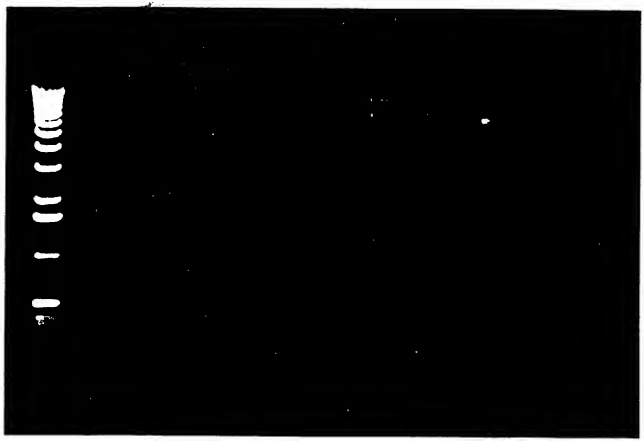

FIGURE 1

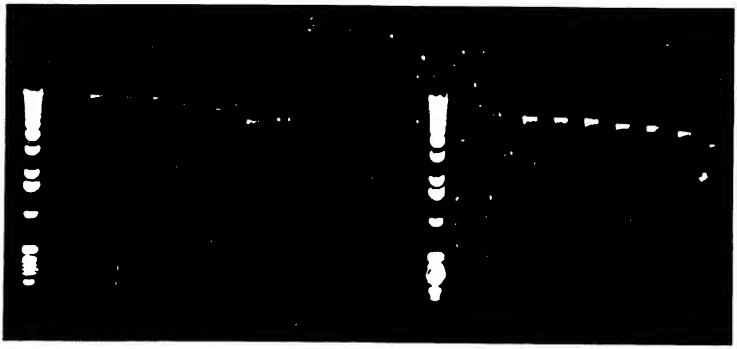

FIGURE 2

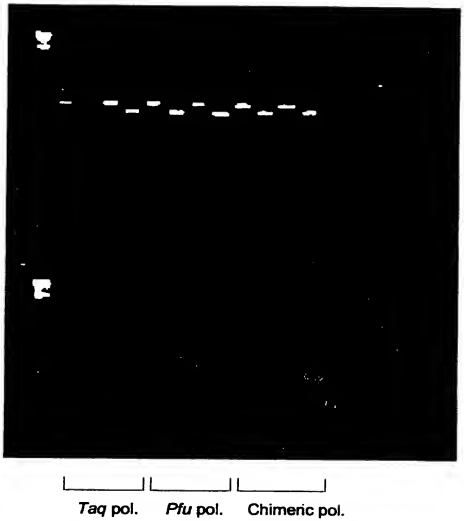
(A)
Wild Type Primer
Extension
(B)
Mutant Primer
Extension
Taq pol.
Pfu pol.
Chimeric pol.

FIGURE 3

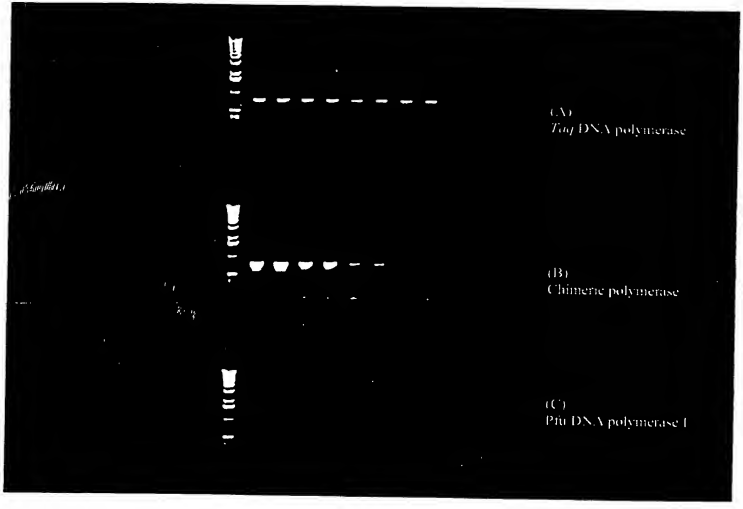


FIGURE 4

SEQUENCE LISTING

<110> Qiagen GmbH
Loffert, Dirk
Missel, Andreas
Kang, Jie

<120> Thermostable Chimeric Nucleic Acid Polymerases and Uses Thereof

<130> PA058-PCT

<140> (not yet assigned)
<141> (concurrently herewith)

<150> US 09/506,153
<151> 2000-02-17

<160> 17

<170> Microsoft Word 97 SR-1

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<400> 1

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Arg Lys Glu Pro Met Trp Ala Asp Leu Leu Ala Leu Ala Ala Ala Arg
        35                  40                  45

Gly Gly Arg Val His Arg Ala Pro Glu Pro Tyr Lys Ala Leu Arg Asp
    50                  55                  60

Leu Lys Glu Ala Arg Gly Leu Leu Ala Lys Asp Leu Ser Val Leu Ala
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65 70 75 80

Leu Arg Glu Gly Leu Gly Leu Pro Pro Gly Asp Asp Pro Met Leu Leu
85 90 95

Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr Pro Glu Gly Val Ala Arg
100 105 110

Arg Tyr Gly Gly Glu Trp Thr Glu Glu Ala Gly Glu Arg Ala Ala Leu
115 120 125

Ser Glu Arg Leu Phe Ala Asn Leu Trp Gly Arg Leu Glu Gly Glu Glu
130 135 140

Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu Arg Pro Leu Ser Ala Val
145 150 155 160

Leu Ala His Met Glu Ala Thr Gly Val Arg Leu Asp Val Ala Tyr Leu
165 170 175

Arg Ala Leu Ser Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala
180 185 190

Glu Val Phe Arg Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp
195 200 205

Gln Leu Glu Arg Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly
210 215 220

Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu
225 230 235 240

Ala Leu Arg Glu Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg
245 250 255

Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu
260 265 270

Ile His Pro Arg Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala
275 280 285

Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile

290 295 300

Pro Val Arg Thr Pro Leu Gly Gln Arg Ile Arg Arg Ala Phe Ile Ala
305 310 315 320

Glu Glu Gly Trp Leu Leu Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu
325 330 335

Arg Val Leu Ala His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe
340 345 350

Gln Glu Gly Arg Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly
355 360 365

Val Pro Arg Glu Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr
370 375 380

Ile Asn Phe Gly Val Leu Tyr Gly Met Ser Ala His Arg Leu Ser Gln
385 390 395 400

Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr
405 410 415

Phe Gln Ser Phe Pro Lys Val Arg Ala Trp Ile Glu Lys Thr Leu Glu
420 425 430

Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg
435 440 445

Tyr Val Pro Asp Leu Glu Ala Arg Val Lys Ser Val Arg Glu Ala Ala
450 455 460

Glu Arg Met Ala Phe Asn Met Pro Val Gln Gly Thr Ala Ala Asp Leu
465 470 475 480

Met Lys Leu Ala Met Val Lys Leu Phe Pro Arg Leu Glu Glu Met Gly
485 490 495

Ala Arg Met Leu Leu Gln Val His Asp Glu Leu Val Leu Glu Ala Pro
500 505 510

Lys Glu Arg Ala Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu

515 520 525

Gly Val Tyr Pro Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly
530 535 540

Glu Asp Trp Leu Ser Ala Lys Glu
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<213> Thermus thermophilus

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20 25 30

Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Pro Glu Pro Met Trp
35 40 45

Ala Glu Leu Lys Ala Leu Ala Ala Cys Arg Asp Gly Arg Val His Arg
50 55 60

Ala Ala Asp Pro Leu Ala Gly Leu Lys Asp Leu Lys Glu Val Arg Gly
65 70 75 80

Leu Leu Ala Lys Asp Leu Ala Val Leu Ala Ser Arg Glu Gly Leu Asp
85 90 95

Leu Val Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro
100 105 110

Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp
115 120 125

Thr Glu Asp Ala Ala His Arg Ala Leu Leu Ser Glu Arg Leu His Arg
130 135 140

Asn Leu Leu Lys Arg Leu Glu Gly Glu Glu Lys Leu Leu Trp Leu Tyr
145 150 155 160

His Glu Val Glu Lys Pro Leu Ser Arg Val Leu Ala His Met Glu Ala
165 170 175

Thr Gly Val Arg Arg Asp Val Ala Tyr Leu Gln Ala Leu Ser Leu Glu
180 185 190

Leu Ala Glu Glu Ile Arg Arg Leu Glu Glu Glu Val Phe Arg Leu Ala
195 200 205

Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu
210 215 220

Phe Asp Glu Leu Arg Leu Pro Ala Leu Gly Lys Thr Gln Lys Thr Gly
225 230 235 240

Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His
245 250 255

Pro Ile Val Glu Lys Ile Leu Gln His Arg Glu Leu Thr Lys Leu Lys
260 265 270

Asn Thr Tyr Val Asp Pro Leu Pro Ser Leu Val His Pro Arg Thr Gly
275 280 285

Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu
290 295 300

Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu
305 310 315 320

Gly Gln Arg Ile Arg Arg Ala Phe Val Ala Glu Ala Gly Trp Ala Leu
325 330 335

Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu
340 345 350

Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Lys Asp Ile
355 360 365

His Thr Gln Thr Ala Ser Trp Met Phe Gly Val Pro Pro Glu Ala Val
370 375 380

Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Val Asn Phe Gly Val Leu
385 390 395 400

Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr
405 410 415

Glu Glu Ala Val Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys
420 425 430

Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Lys Arg Gly
435 440 445

Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Asn
450 455 460

Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn
465 470 475 480

Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val
485 490 495

Lys Leu Phe Pro Arg Leu Arg Glu Met Gly Ala Arg Met Leu Leu Gln
500 505 510

Val His Asp Glu Leu Leu Leu Glu Ala Pro Gln Ala Arg Ala Glu Glu
515 520 525

Val Ala Ala Leu Ala Lys Glu Ala Met Glu Lys Ala Tyr Pro Leu Ala
530 535 540

Val Pro Leu Glu Val Glu Val Gly Met Gly Glu Asp Trp Leu Ser Ala
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<211> 396

<212> PRT
<213> Pyrococcus horikoshii

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Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Val Glu Tyr Asp Arg
20 25 30

Asn Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Ala Ile
35 40 45

Asp Glu Ile Lys Lys Ile Thr Ala Gln Arg His Gly Lys Val Val Arg
50 55 60

Ile Val Glu Thr Glu Lys Ile Gln Arg Lys Phe Leu Gly Arg Pro Ile
65 70 75 80

Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile
85 90 95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Thr Pro
115 120 125

Met Glu Gly Asn Glu Lys Leu Thr Phe Leu Ala Val Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Val Ile Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Glu Gly Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190

Arg Leu Ile Arg Val Ile Lys Glu Lys Asp Pro Asp Val Ile Ile Thr
195 200 205

Tyr Asn Gly Asp Asn Phe Asp Phe Pro Tyr Leu Leu Lys Arg Ala Glu
210 215 220

Lys Leu Gly Ile Lys Leu Leu Leu Gly Arg Asp Asn Ser Glu Pro Lys
225 230 235 240

Met Gln Lys Met Gly Asp Ser Leu Ala Val Glu Ile Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Phe Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
305 310 315 320

Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ala Arg Leu
325 330 335

Val Gly Gln Pro Val Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
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Pro Asn Lys Pro Asp Glu Lys Glu Tyr Glu Arg Arg Leu Arg Glu Ser
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Tyr Glu Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly
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35 40 45

Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
50 55 60

Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
65 70 75 80

Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95

Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125

Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile
165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190

Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr
195 200 205

Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu

210 215 220

Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240

Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320

Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu
325 330 335

Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala
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Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser
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Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly
385 390 395

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<211> 421
<212> PRT
<213> Pyrococcus woesei

<400> 5

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20 25 30

Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Ile
35 40 45

Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
50 55 60

Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
65 70 75 80

Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95

Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125

Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile
165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190

Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr
195 200 205

Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220

Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240

Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320

Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu
325 330 335

Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala
355 360 365

Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser
370 375 380

Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400

Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415

His Asn Val Ser Pro
420

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<211> 508

<212> PRT
<213> Sulfolobus solfataricus

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Glu Lys Lys Val Val Arg Arg Glu Trp Leu Glu Glu Ala Gln Glu Asn
35 40 45

Lys Ile Tyr Phe Leu Leu Gln Val Asp Tyr Asp Gly Lys Lys Gly Lys
50 55 60

Ala Val Cys Lys Leu Phe Asp Lys Glu Thr Gln Lys Ile Tyr Ala Leu
65 70 75 80

Tyr Asp Asn Thr Gly His Lys Pro Tyr Phe Leu Val Asp Leu Glu Pro
85 90 95

Asp Lys Val Gly Lys Ile Pro Lys Ile Val Arg Asp Pro Ser Phe Asp
100 105 110

His Ile Glu Thr Val Ser Lys Ile Asp Pro Tyr Thr Trp Asn Lys Phe
115 120 125

Lys Leu Thr Lys Ile Val Val Arg Asp Pro His Ala Val Arg Arg Leu
130 135 140

Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His Ile Lys Tyr Phe Asn
145 150 155 160

Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly Met Pro Tyr Val Val
165 170 175

Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser Leu Asp Glu Lys Asp
180 185 190

Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser Asp Glu Met Thr Arg
195 200 205

Gln Met Ala Val Asp Trp Leu Pro Ile Phe Glu Thr Glu Ile Pro Lys
210 215 220

Ile Lys Arg Val Ala Ile Asp Ile Glu Val Tyr Thr Pro Val Lys Gly
225 230 235 240

Arg Ile Pro Asp Ser Gln Lys Ala Glu Phe Pro Ile Ile Ser Ile Ala
245 250 255

Leu Ala Gly Ser Asp Gly Leu Lys Lys Val Leu Val Leu Asn Arg Asn
260 265 270

Asp Val Asn Glu Gly Ser Val Lys Leu Asp Gly Ile Ser Val Glu Arg
275 280 285

Phe Asn Thr Glu Tyr Glu Leu Leu Gly Arg Phe Phe Asp Ile Leu Leu
290 295 300

Glu Tyr Pro Ile Val Leu Thr Phe Asn Gly Asp Asp Phe Asp Leu Pro
305 310 315 320

Tyr Ile Tyr Phe Arg Ala Leu Lys Leu Gly Tyr Phe Pro Glu Glu Ile
325 330 335

Pro Ile Asp Val Ala Gly Lys Asp Glu Ala Lys Tyr Leu Ala Gly Leu
340 345 350

His Ile Asp Leu Tyr Lys Phe Phe Phe Asn Lys Ala Val Arg Asn Tyr
355 360 365

Ala Phe Glu Gly Lys Tyr Asn Glu Tyr Asn Leu Asp Ala Val Ala Lys
370 375 380

Ala Leu Leu Gly Thr Ser Lys Val Lys Val Asp Thr Leu Ile Ser Phe
385 390 395 400

Leu Asp Val Glu Lys Leu Ile Glu Tyr Asn Phe Arg Asp Ala Glu Ile
405 410 415

Thr Leu Gln Leu Thr Thr Phe Asn Asn Asp Leu Thr Met Lys Leu Ile
420 425 430

Val Leu Phe Ser Arg Ile Ser Arg Leu Gly Ile Glu Glu Leu Thr Arg
435 440 445

Thr Glu Ile Ser Thr Trp Val Lys Asn Leu Tyr Tyr Trp Glu His Arg
450 455 460

Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu Ile Leu Ala Lys Ser
465 470 475 480

Ser Asn Ile Arg Thr Ser Ala Leu Ile Lys Gly Lys Gly Tyr Lys Gly
485 490 495

Ala Val Val Ile Asp Pro Pro Ala Gly Ile Phe Phe
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<213> Thermus aquaticus

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Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp
35 40 45

Ala Asp Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg
50 55 60

Ala Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly
65 70 75 80

Leu Leu Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly
85 90 95

Leu Pro Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro

100 105 110

Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp
115 120 125

Thr Glu Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala
130 135 140

Asn Leu Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr
145 150 155 160

Arg Glu Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala
165 170 175

Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu
180 185 190

Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala
195 200 205

Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu
210 215 220

Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly
225 230 235 240

Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His
245 250 255

Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys
260 265 270

Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly
275 280 285

Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu
290 295 300

Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu
305 310 315 320

Gly Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu

325 330 335

Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu
340 345 350

Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile
355 360 365

His Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val
370 375 380

Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu
385 390 395 400

Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr
405 410 415

Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys
420 425 430

Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly
435 440 445

Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu
450 455 460

Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn
465 470 475 480

Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val
485 490 495

Lys Leu Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln
500 505 510

Val His Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala
515 520 525

Val Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala
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Val Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala

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Lys Glu

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<211> 958
<212> PRT
<213> Artificial Sequence

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20 25 30

Asn Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Ala Ile
35 40 45

Asp Glu Ile Lys Lys Ile Thr Ala Gln Arg His Gly Lys Val Val Arg
50 55 60

Ile Val Glu Thr Glu Lys Ile Gln Arg Lys Phe Leu Gly Arg Pro Ile
65 70 75 80

Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile
85 90 95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Thr Pro
115 120 125

Met Glu Gly Asn Glu Lys Leu Thr Phe Leu Ala Val Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Val Ile Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Glu Gly Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190

Arg Leu Ile Arg Val Ile Lys Glu Lys Asp Pro Asp Val Ile Ile Thr
195 200 205

Tyr Asn Gly Asp Asn Phe Asp Phe Pro Tyr Leu Leu Lys Arg Ala Glu
210 215 220

Lys Leu Gly Ile Lys Leu Leu Leu Gly Arg Asp Asn Ser Glu Pro Lys
225 230 235 240

Met Gln Lys Met Gly Asp Ser Leu Ala Val Glu Ile Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Phe Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
305 310 315 320

Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ala Arg Leu
325 330 335

Val Gly Gln Pro Val Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365

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Pro Asn Lys Pro Asp Glu Lys Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380

Tyr Glu Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Ala Phe Leu Glu
385                 390                 395                 400

Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu Glu Ser
                405                 410                 415

Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly Ala Phe
            420                 425                 430

Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp Leu Leu
        435                 440                 445

Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro Glu Pro
    450                 455                 460

Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu Ala Lys
465                 470                 475                 480

Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro Pro Gly
                485                 490                 495

Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr
            500                 505                 510

Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu Glu Ala
        515                 520                 525

Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu Trp Gly
    530                 535                 540

Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu
545                 550                 555                 560

Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly Val Arg
                565                 570                 575

Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu
            580                 585                 590
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Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe
595 600 605

Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu
610 615 620

Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr
625 630 635 640

Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu
645 650 655

Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile
660 665 670

Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr
675 680 685

Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp
690 695 700

Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile
705 710 715 720

Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp
725 730 735

Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu
740 745 750

Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr
755 760 765

Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met
770 775 780

Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser
785 790 795 800

Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln
805 810 815

Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp
820 825 830

Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr
835 840 845

Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys
850 855 860

Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln
865 870 875 880

Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro
885 890 895

Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu
900 905 910

Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu
915 920 925

Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu
930 935 940

Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
945 950 955

<210> 9
<211> 958
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Pwo/Taq
Chimeric polymerase

<400> 9
Met Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15

Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp Arg
20 25 30

Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Ile
35 40 45

Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
50 55 60

Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
65 70 75 80

Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95

Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125

Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile
165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190

Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr
195 200 205

Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220

Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240

Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320

Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu
325 330 335

Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala
355 360 365

Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser
370 375 380

Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Ala Phe Leu Glu
385 390 395 400

Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu Glu Ser
405 410 415

Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly Ala Phe
420 425 430

Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp Leu Leu
435 440 445

Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro Glu Pro
450 455 460

Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu Ala Lys
465 470 475 480

Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro Pro Gly
485 490 495

Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr
500 505 510

Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu Glu Ala
515 520 525

Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu Trp Gly
530 535 540

Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu
545 550 555 560

Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly Val Arg
565 570 575

Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu
580 585 590

Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe
595 600 605

Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu
610 615 620

Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr
625 630 635 640

Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu
645 650 655

Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile
660 665 670

Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr
675 680 685

```
Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp
    690                 695                 700

Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile
705                 710                 715                 720

Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp
                725                 730                 735

Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu
            740                 745                 750

Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr
        755                 760                 765

Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met
    770                 775                 780

Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser
785                 790                 795                 800

Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln
                805                 810                 815

Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp
            820                 825                 830

Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr
        835                 840                 845

Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys
    850                 855                 860

Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln
865                 870                 875                 880

Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro
                885                 890                 895

Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu
            900                 905                 910
```

Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu
915 920 925

Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu
930 935 940

Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
945 950 955

<210> 10
<211> 1060
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Sso/Taq Chimeric polymerase

<400> 10
Met Thr Lys Gln Leu Thr Leu Phe Asp Ile Pro Ser Ser Lys Pro Ala
1 5 10 15

Lys Ser Glu Gln Asn Thr Gln Gln Ser Gln Gln Ser Ala Pro Val Glu
20 25 30

Glu Lys Lys Val Val Arg Arg Glu Trp Leu Glu Glu Ala Gln Glu Asn
35 40 45

Lys Ile Tyr Phe Leu Leu Gln Val Asp Tyr Asp Gly Lys Lys Gly Lys
50 55 60

Ala Val Cys Lys Leu Phe Asp Lys Glu Thr Gln Lys Ile Tyr Ala Leu
65 70 75 80

Tyr Asp Asn Thr Gly His Lys Pro Tyr Phe Leu Val Asp Leu Glu Pro
85 90 95

Asp Lys Val Gly Lys Ile Pro Lys Ile Val Arg Asp Pro Ser Phe Asp
100 105 110

His Ile Glu Thr Val Ser Lys Ile Asp Pro Tyr Thr Trp Asn Lys Phe

115 120 125

Lys Leu Thr Lys Ile Val Val Arg Asp Pro His Ala Val Arg Arg Leu
130 135 140

Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His Ile Lys Tyr Phe Asn
145 150 155 160

Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly Met Pro Tyr Val Val
165 170 175

Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser Leu Asp Glu Lys Asp
180 185 190

Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser Asp Glu Met Thr Arg
195 200 205

Gln Met Ala Val Asp Trp Leu Pro Ile Phe Glu Thr Glu Ile Pro Lys
210 215 220

Ile Lys Arg Val Ala Ile Asp Ile Glu Val Tyr Thr Pro Val Lys Gly
225 230 235 240

Arg Ile Pro Asp Ser Gln Lys Ala Glu Phe Pro Ile Ile Ser Ile Ala
245 250 255

Leu Ala Gly Ser Asp Gly Leu Lys Lys Val Leu Val Leu Asn Arg Asn
260 265 270

Asp Val Asn Glu Gly Ser Val Lys Leu Asp Gly Ile Ser Val Glu Arg
275 280 285

Phe Asn Thr Glu Tyr Glu Leu Leu Gly Arg Phe Phe Asp Ile Leu Leu
290 295 300

Glu Tyr Pro Ile Val Leu Thr Phe Asn Gly Asp Asp Phe Asp Leu Pro
305 310 315 320

Tyr Ile Tyr Phe Arg Ala Leu Lys Leu Gly Tyr Phe Pro Glu Glu Ile
325 330 335

Pro Ile Asp Val Ala Gly Lys Asp Glu Ala Lys Tyr Leu Ala Gly Leu

340 345 350

His Ile Asp Leu Tyr Lys Phe Phe Phe Asn Lys Ala Val Arg Asn Tyr
355 360 365

Ala Phe Glu Gly Lys Tyr Asn Glu Tyr Asn Leu Asp Ala Val Ala Lys
370 375 380

Ala Leu Leu Gly Thr Ser Lys Val Lys Val Asp Thr Leu Ile Ser Phe
385 390 395 400

Leu Asp Val Glu Lys Leu Ile Glu Tyr Asn Phe Arg Asp Ala Glu Ile
405 410 415

Thr Leu Gln Leu Thr Thr Phe Asn Asn Asp Leu Thr Met Lys Leu Ile
420 425 430

Val Leu Phe Ser Arg Ile Ser Arg Leu Gly Ile Glu Glu Leu Thr Arg
435 440 445

Thr Glu Ile Ser Thr Trp Val Lys Asn Leu Tyr Tyr Trp Glu His Arg
450 455 460

Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu Ile Leu Ala Lys Ser
465 470 475 480

Ser Asn Ile Arg Thr Ser Ala Leu Ile Lys Gly Lys Gly Tyr Lys Gly
485 490 495

Ala Val Val Ile Asp Pro Pro Ala Gly Ile Phe Phe Leu Leu His Glu
500 505 510

Phe Gly Leu Leu Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro
515 520 525

Pro Pro Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro
530 535 540

Met Trp Ala Asp Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val
545 550 555 560

His Arg Ala Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala

565 570 575

Arg Gly Leu Leu Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly
580 585 590

Leu Gly Leu Pro Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu
595 600 605

Asp Pro Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly
610 615 620

Glu Trp Thr Glu Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu
625 630 635 640

Phe Ala Asn Leu Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp
645 650 655

Leu Tyr Arg Glu Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met
660 665 670

Glu Ala Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser
675 680 685

Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg
690 695 700

Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg
705 710 715 720

Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys
725 730 735

Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu
740 745 750

Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys
755 760 765

Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg
770 775 780

Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly

785 790 795 800

Arg Leu Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr
805 810 815

Pro Leu Gly Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp
820 825 830

Leu Leu Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala
835 840 845

His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg
850 855 860

Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu
865 870 875 880

Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly
885 890 895

Val Leu Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile
900 905 910

Pro Tyr Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe
915 920 925

Pro Lys Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg
930 935 940

Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp
945 950 955 960

Leu Glu Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala
965 970 975

Phe Asn Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala
980 985 990

Met Val Lys Leu Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu
995 1000 1005

Leu Gln Val His Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala

1010 1015 1020

Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro
1025 1030 1035 1040

Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu
1045 1050 1055

Ser Ala Lys Glu
1060

<210> 11
<211> 983
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Pwo/Taq
Chimeric polymerase

<400> 11
Met Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15

Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp Arg
20 25 30

Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Ile
35 40 45

Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
50 55 60

Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
65 70 75 80

Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95

Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125

Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile
165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190

Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr
195 200 205

Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220

Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240

Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320

Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu
325 330 335

```
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala
        355                 360                 365

Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser
    370                 375                 380

Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400

Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415

His Asn Val Ser Pro Ala Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu
            420                 425                 430

Leu His Glu Phe Gly Leu Leu Glu Ser Pro Lys Ala Leu Glu Glu Ala
        435                 440                 445

Pro Trp Pro Pro Pro Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg
    450                 455                 460

Lys Glu Pro Met Trp Ala Asp Leu Leu Ala Leu Ala Ala Ala Arg Gly
465                 470                 475                 480

Gly Arg Val His Arg Ala Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu
                485                 490                 495

Lys Glu Ala Arg Gly Leu Leu Ala Lys Asp Leu Ser Val Leu Ala Leu
            500                 505                 510

Arg Glu Gly Leu Gly Leu Pro Pro Gly Asp Asp Pro Met Leu Leu Ala
        515                 520                 525

Tyr Leu Leu Asp Pro Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg
    530                 535                 540

Tyr Gly Gly Glu Trp Thr Glu Glu Ala Gly Glu Arg Ala Ala Leu Ser
545                 550                 555                 560
```

Glu Arg Leu Phe Ala Asn Leu Trp Gly Arg Leu Glu Gly Glu Glu Arg
565 570 575

Leu Leu Trp Leu Tyr Arg Glu Val Glu Arg Pro Leu Ser Ala Val Leu
580 585 590

Ala His Met Glu Ala Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Arg
595 600 605

Ala Leu Ser Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu
610 615 620

Val Phe Arg Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln
625 630 635 640

Leu Glu Arg Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys
645 650 655

Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala
660 665 670

Leu Arg Glu Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu
675 680 685

Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile
690 695 700

His Pro Arg Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr
705 710 715 720

Ala Thr Gly Arg Leu Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro
725 730 735

Val Arg Thr Pro Leu Gly Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu
740 745 750

Glu Gly Trp Leu Leu Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg
755 760 765

Val Leu Ala His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln
770 775 780

```
Glu Gly Arg Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly Val
785                 790                 795                 800

Pro Arg Glu Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Ile
                805                 810                 815

Asn Phe Gly Val Leu Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu
            820                 825                 830

Leu Ala Ile Pro Tyr Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe
        835                 840                 845

Gln Ser Phe Pro Lys Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu
    850                 855                 860

Gly Arg Arg Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr
865                 870                 875                 880

Val Pro Asp Leu Glu Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu
                885                 890                 895

Arg Met Ala Phe Asn Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met
            900                 905                 910

Lys Leu Ala Met Val Lys Leu Phe Pro Arg Leu Glu Glu Met Gly Ala
        915                 920                 925

Arg Met Leu Leu Gln Val His Asp Glu Leu Val Leu Glu Ala Pro Lys
    930                 935                 940

Glu Arg Ala Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu Gly
945                 950                 955                 960

Val Tyr Pro Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly Glu
                965                 970                 975

Asp Trp Leu Ser Ala Lys Glu
            980
```

<210> 12

<211> 958
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Pho/Tth Chimeric polymerase

<400> 12

```
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Ile Ile
  1               5                  10                  15

Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Val Glu Tyr Asp Arg
            20                  25                  30

Asn Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Ala Ile
        35                  40                  45

Asp Glu Ile Lys Lys Ile Thr Ala Gln Arg His Gly Lys Val Val Arg
    50                  55                  60

Ile Val Glu Thr Glu Lys Ile Gln Arg Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80

Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile
                85                  90                  95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Thr Pro
        115                 120                 125

Met Glu Gly Asn Glu Lys Leu Thr Phe Leu Ala Val Asp Ile Glu Thr
    130                 135                 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Val Ile Met Ile
145                 150                 155                 160

Ser Tyr Ala Asp Glu Glu Gly Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
```

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190

Arg Leu Ile Arg Val Ile Lys Glu Lys Asp Pro Asp Val Ile Ile Thr
195 200 205

Tyr Asn Gly Asp Asn Phe Asp Phe Pro Tyr Leu Leu Lys Arg Ala Glu
210 215 220

Lys Leu Gly Ile Lys Leu Leu Leu Gly Arg Asp Asn Ser Glu Pro Lys
225 230 235 240

Met Gln Lys Met Gly Asp Ser Leu Ala Val Glu Ile Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Phe Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
305 310 315 320

Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ala Arg Leu
325 330 335

Val Gly Gln Pro Val Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365

Pro Asn Lys Pro Asp Glu Lys Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380

Tyr Glu Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Ala Phe Leu Glu
385 390 395 400

Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu Glu Ala
405 410 415

Pro Ala Pro Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly Ala Phe
420 425 430

Val Gly Phe Val Leu Ser Arg Pro Glu Pro Met Trp Ala Glu Leu Lys
435 440 445

Ala Leu Ala Ala Cys Arg Asp Gly Arg Val His Arg Ala Ala Asp Pro
450 455 460

Leu Ala Gly Leu Lys Asp Leu Lys Glu Val Arg Gly Leu Leu Ala Lys
465 470 475 480

Asp Leu Ala Val Leu Ala Ser Arg Glu Gly Leu Asp Leu Val Pro Gly
485 490 495

Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr
500 505 510

Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu Asp Ala
515 520 525

Ala His Arg Ala Leu Leu Ser Glu Arg Leu His Arg Asn Leu Leu Lys
530 535 540

Arg Leu Glu Gly Glu Glu Lys Leu Leu Trp Leu Tyr His Glu Val Glu
545 550 555 560

Lys Pro Leu Ser Arg Val Leu Ala His Met Glu Ala Thr Gly Val Arg
565 570 575

Arg Asp Val Ala Tyr Leu Gln Ala Leu Ser Leu Glu Leu Ala Glu Glu
580 585 590

Ile Arg Arg Leu Glu Glu Glu Val Phe Arg Leu Ala Gly His Pro Phe
595 600 605

Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu
610 615 620

Arg Leu Pro Ala Leu Gly Lys Thr Gln Lys Thr Gly Lys Arg Ser Thr
625 630 635 640

Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu
645 650 655

Lys Ile Leu Gln His Arg Glu Leu Thr Lys Leu Lys Asn Thr Tyr Val
660 665 670

Asp Pro Leu Pro Ser Leu Val His Pro Arg Thr Gly Arg Leu His Thr
675 680 685

Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp
690 695 700

Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile
705 710 715 720

Arg Arg Ala Phe Val Ala Glu Ala Gly Trp Ala Leu Val Ala Leu Asp
725 730 735

Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu
740 745 750

Asn Leu Ile Arg Val Phe Gln Glu Gly Lys Asp Ile His Thr Gln Thr
755 760 765

Ala Ser Trp Met Phe Gly Val Pro Pro Glu Ala Val Asp Pro Leu Met
770 775 780

Arg Arg Ala Ala Lys Thr Val Asn Phe Gly Val Leu Tyr Gly Met Ser
785 790 795 800

Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Val
805 810 815

Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp
820 825 830

Ile Glu Lys Thr Leu Glu Glu Gly Arg Lys Arg Gly Tyr Val Glu Thr
835 840 845

Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Asn Ala Arg Val Lys
850 855 860

Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln
865 870 875 880

Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro
885 890 895

Arg Leu Arg Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu
900 905 910

Leu Leu Leu Glu Ala Pro Gln Ala Arg Ala Glu Glu Val Ala Ala Leu
915 920 925

Ala Lys Glu Ala Met Glu Lys Ala Tyr Pro Leu Ala Val Pro Leu Glu
930 935 940

Val Glu Val Gly Met Gly Glu Asp Trp Leu Ser Ala Lys Gly
945 950 955

<210> 13
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer sequence

<400> 13
tttcccagtc acgacgttgt aaaacgacgg
30

<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer sequence

<400> 14
gcaccccgct tgggcagag
19

<210> 15
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer sequence

<400> 15
tcccgcccct cctggaagac
20

<210> 16
<211> 395
<212> PRT
<213> Thermococcus pacificus

<400> 16

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| Met | Ile | Leu | Asp | Ala | Asp | Tyr | Ile | Thr | Glu | Asp | Gly | Lys | Pro | Val | Ile |
| 1 | | | | 5 | | | | 10 | | | | | | 15 | |
| Arg | Ile | Phe | Arg | Lys | Glu | Lys | Gly | Glu | Phe | Lys | Ile | Glu | Tyr | Asp | Arg |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Asn | Phe | Glu | Pro | Tyr | Phe | Tyr | Ala | Leu | Leu | Lys | Asp | Asp | Ser | Ala | Ile |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Glu | Asp | Val | Lys | Lys | Ile | Thr | Ala | Glu | Arg | His | Gly | Thr | Thr | Val | Arg |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Val | Val | Arg | Ala | Glu | Lys | Val | Lys | Lys | Lys | Phe | Leu | Gly | Arg | Pro | Ile |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile
85 90 95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Tyr Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125

Met Glu Gly Asp Glu Glu Leu Lys Met Leu Ala Phe Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Ala Glu Gly Pro Ile Leu Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Ile
165 170 175

Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
180 185 190

Arg Phe Leu Arg Val Ile Lys Glu Lys Asp Pro Asp Val Leu Ile Thr
195 200 205

Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu
210 215 220

Lys Leu Gly Val Lys Phe Ile Leu Gly Arg Asp Gly Ser Glu Pro Lys
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Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
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275 280 285

Lys Val Tyr Ala Glu Glu Ile Thr Gln Ala Trp Glu Thr Gly Glu Gly
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Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
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Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
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Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
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Asn Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
35 40 45

Glu Asp Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Thr Val Arg
50 55 60

Val Val Arg Ala Glu Lys Val Lys Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile
85 90 95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Tyr Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125

Met Glu Gly Asp Glu Glu Leu Lys Met Leu Ala Phe Asp Ile Glu Thr
130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Ala Glu Gly Pro Ile Leu Met Ile
145 150 155 160

Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Ile
165 170 175

Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
180 185 190

Arg Phe Leu Arg Val Ile Lys Glu Lys Asp Pro Asp Val Leu Ile Thr
195 200 205

Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu
210 215 220

Lys Leu Gly Val Lys Phe Ile Leu Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240

Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
245 250 255

His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Gln Pro Lys Glu
275 280 285

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                325                 330                 335

Val Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365

Pro Asn Lys Pro Asp Glu Lys Glu Leu Ala Arg Arg Arg Glu Ser Tyr
    370                 375                 380

Ala Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Ala Phe Leu Glu Arg
385                 390                 395                 400

Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu Glu Ser Pro
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Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly Ala Phe Val
            420                 425                 430

Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp Leu Leu Ala
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Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro Glu Pro Tyr
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Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu Ala Lys Asp
465                 470                 475                 480

Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro Pro Gly Asp
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Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr Pro
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Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu Glu Ala Gly
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Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu Trp Gly Arg
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Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu Arg
545                 550                 555                 560

Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly Val Arg Leu
                565                 570                 575

Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu Ile
            580                 585                 590

Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe Asn
        595                 600                 605

Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu Gly
    610                 615                 620

Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser
625                 630                 635                 640

Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu Lys
                645                 650                 655

Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp
            660                 665                 670

Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr Arg
        675                 680                 685

Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp Pro
    690                 695                 700

Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile Arg
705                 710                 715                 720

Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp Tyr
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Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu Asn
            740                 745                 750

Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr Ala
        755                 760                 765

Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met Arg
    770                 775                 780

Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser Ala
785                 790                 795                 800

His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln Ala
                805                 810                 815

Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp Ile
            820                 825                 830

Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr Leu
        835                 840                 845

Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys Ser
    850                 855                 860

Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln Gly
865                 870                 875                 880

Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro Arg
                885                 890                 895

Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu Leu
            900                 905                 910

Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu Ala
        915                 920                 925

Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu Val
    930                 935                 940

Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
945                 950                 955
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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Filed on 17 February 2000 (17.02.2000)

(71) Applicant *(for all designated States except US)*: QIAGEN GMBH [DE/DE]; Max-Volmer-Strasse 4, 40724 Hilden (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LÖFFERT, Dirk [DE/DE]; Bahlenstrasse 8, 40589 Düsseldorf (DE). MISSEL, Andreas [DE/DE]; Kirchfeldstrasse 93, 40215 Düsseldorf (DE). KANG, Jie [DE/DE]; Meisenweg 25, 40822 Mettmann (DE).

(74) Common Representative: QIAGEN GMBH; Max-Volmer-Strasse 4, 40724 Hilden (DE).

(81) Designated States *(national)*: JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *with international search report*

(88) Date of publication of the international search report:
25 April 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: THERMOSTABLE CHIMERIC NUCLEIC ACID POLYMERASES AND USES THEREOF

(57) Abstract: Novel thermostable chimeric nucleic acid polymerases and methods for their generation and use are disclosed. It is shown that these chimeric nucleic acid polymerases, such as DNA polymerases, can be constructed using enzymatically active domains, isolated from different proteins or chemically synthesized. It is demonstrated that chimeric nucleic acid polymerases of the present invention possess the chemical and physical properties of their component domains (e.g., exonuclease activity, thermostability) and that the chimeric polymerases are thermostable.

WO 01/61015 A3

# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 01/01790

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12N15/54 C12N15/62 C12N15/70 C12N9/12 C12N1/21
C12Q1/68 C12P19/34

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, SEQUENCE SEARCH, BIOSIS, EPO-Internal

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 99 47649 A (ANKENBAUER WALTRAUD ;SCHOMBURG DIETMAR (DE); VILLBRANDT BRITTA (DE) 23 September 1999 (1999-09-23) the whole document --- | 1-10, 36-43, 69-81 |
| X | EP 0 892 058 A (HOFFMANN LA ROCHE) 20 January 1999 (1999-01-20) the whole document --- | 1-9, 36-42, 69-81 |
| X | WO 97 29209 A (HARVARD COLLEGE) 14 August 1997 (1997-08-14) the whole document --- -/-- | 1,2,6-8, 36,37, 39-41, 69-81 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 3 October 2001 | 18. 12. 2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | HORNIG H. |

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 92 06200 A (CETUS CORP)<br>16 April 1992 (1992-04-16)<br><br>page 27, line 9 -page 28, line 7; claim 16<br>--- | 1-10,<br>36-43,<br>69-81 |
| P,X | GB 2 344 591 A (BIOLINE LIMITED)<br>14 June 2000 (2000-06-14)<br><br>claims 1-23<br>--- | 1-11,<br>36-44,<br>69-81 |
| E | WO 01 18213 A (DZIEGLEWSKA HANNA<br>;KRISTENSEN TOM (NO))<br>15 March 2001 (2001-03-15)<br>claims 1-20<br>----- | 1-11,<br>36-44,<br>69-81 |

# INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/EP 01/01790

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

11, 44 completely; (1-10,36-43,69-81 partly)

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (11,44)-completely, (1-10,36-43,69-81)-partially

A chimeric nucleic acid polymerase comprising at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, and said chimeric nucleic acid polymerase is thermostable; said polymerase comprises a 3'-5' exonuclease domain and a 5'-3' polymerase domain; said polymerase domain selected from Taq DNA polymerase comprising a Stoffel fragment of Taq polymerase or a mutant, variant, or derivative thereof; an isolated polynucleotide encoding said thermostable chimeric nucleic acid polymerase; a vector comprising said nuclec acid; a recombinant host cell transformed with said vector; a kit for polymerization comprising said chimeric nucleic acid polymerase; a process for nucleic acid polymerization using said chimeric polymerase; a process for producing said thermostable chimeric nucleic acid polymerase;

2. Claims: (12,24-30,45,57-63)-completely, (1-10,35-43, 68-81)-partially

Idem as invention 1 but limited to a 5'-3' polymerase domain of Taq DNA polymerase comprising amino acid residues 281-832 (SEQ ID No. 1); and wherein the chimeric polymerase are selected from: (i) 5'-3' polymerase domain from amino acid residues 281 to 832 of Taq DNA polymerase (SEQ ID No. 1), and 3'-5' exonuclease domain comprising amino acid residues 1-396 of Pho DNA polymerase (SEQ ID No. 3); (ii) 5'-3' polymerase domain from amino acid residues 281 to 832 of Taq DNA polymerase (SEQ ID No. 1), and 3'-5' exonuclease domain comprising amino acid residues 1-396 and/or residues 1-421 of Pwo DNA polymerase (SEQ ID Nos. 4 or 5); (iii) 5'-3' polymerase domain from amino acid residues 281 to 832 of Taq DNA polymerase (SEQ ID No. 1), and 3'-5' exonuclease domain comprises amino acid residues 1-508 of Sso DNA polymerase (SEQ ID No. 6) resulting in SEQ ID No. 10;

3. Claims: (13,31,32,46,64,65)-completely, (1-10,35-43, 68-81)-partially

Idem as invention 1 but limited to a 5'-3' polymerase domain of Taq DNA polymerase comprising amino acid residues 271-832 (SEQ ID No. 7); and wherein the chimeric polymerase are selected from: (i) 5'-3' polymerase domain from amino acid residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-396 of Pho DNA polymerase (SEQ ID No. 3) resulting in SEQ ID No.8; (ii) 5'-3' polymerase domain from amino acid

residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-396 of Pwo DNA polymerase (SEQ ID No. 4) resulting in SEQ ID No.9; (iii) 5'-3' polymerase domain from amino acid residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-395 of Tpac DNA polymerase (SEQ ID No. 16) resulting in SEQ ID No.17; (iv) 5'-3' polymerase domain from amino acid residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-421 of Pwo DNA polymerase (SEQ ID No. 5) resulting in SEQ ID No.11;

4. Claims: (14,33,34,47,66,67)-completely, (1-10,35-43, 68-81)-partially

Idem as invention 1 but limited to a 5'-3' polymerase domain of Tth DNA polymerase comprising amino acid residues 273-834 (SEQ ID No. 2); and wherein the chimeric polymerase are selected from: (i) 5'-3' polymerase domain from amino acid residues 273 to 834 of Tth DNA polymerase (SEQ ID No. 2), and 3'-5' exonuclease domain comprises amino acid residues 1-396 of Pho DNA polymerase (SEQ ID No. 3) resulting in SEQ ID No.12;

5. Claims: (15,16,48,49)-completely, (1-9,36-43, 69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from Pyrococcus horikoshii Pho DNA polymerase, and SEQ ID No. 3; and insofar as not covered by the other group of inventions;

6. Claims: (17-19,50-52)-completely, (1-9,35,36-43, 69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from Pyrococcus woesei Pwo DNA polymerase, and SEQ ID No. 4 and 5 and insofar as not covered by the other group of inventions;

7. Claims: (20,21,53,54)-completely, (1-9,35,36-43, 69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from Sulfolobus solfataricus Sso DNA polymerase, and SEQ ID No. 6; and insofar as not covered by the other group of inventions;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. Claims: (22,23,55,56)-completely, (1-9,35,36-43, 69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from Thermococcus pacificus Tpac DNA polymerase, and SEQ ID No. 16; and insofar as not covered by the other group of inventions;

# INTERNATIONAL SEARCH REPORT

.ormation on patent family members

Interr nal Application No
PCT/EP 01/01790

| Patent document cited in search report | | Publication date | Patent family member(s) | | | Publication date |
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| WO 9947649 | A | 23-09-1999 | DE | 19810879 | A1 | 16-09-1999 |
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| | | | JP | 10004985 | A | 13-01-1998 |
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| | | | JP | 10000095 | A | 06-01-1998 |
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| | | | AU | 8907791 | A | 28-04-1992 |
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| | | | DK | 550696 | T3 | 19-03-2001 |
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